THE EFFECTS OF DEFOLIATION ON SEASONAL GROWTH DYNAMICS, THE IMPORTANCE OF INTERNAL NITROGEN–RECYCLING AND THE AVAILABILITY OF SOIL NUTRIENTS: IMPLICATIONS FOR THE INVASIVE POTENTIAL OF *BUDDLEIA DAVIDII* (FRANCH.)

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ABBREVIATIONS

A	leaf area
A_e	cumulative emergence leaf area
A_e/ W_d	leaf area ratio
A_l	natural leaf loss
A_{max}	maximum photosynthetic rate at saturated light
A_n	cumulative net leaf area
ANOVA	analysis of Variance
A_p	plant leaf area
B	B. davidii; buddleia
C	C. arborea; coriaria
c_i	intercellular CO2 -concentration
c_i/ c_a	ratio of extracellular and intracellular CO2 -concentration
d	number of days
ε	light use efficiency
G	cumulative growing degree days
G_{95}	cumulative growing degree days to reach the 95% of final leaf area
GLM	generalised linear model
G_{max}	cumulative growing degree days to reach the maximum rate leaf area growth
Gr	cumulative germination
Gr_{max}	maximum number of germinated seeds
g_s	stomatal conductance
J_{max}	maximum photosynthetic electron transport rate
L_l	leaf length

L_w	leaf width
N	nitrogen
^{14}N	source of nitrogen, including natural abundance of 15N only (0.3663 atom ‰)
^{15}N	source of nitrogen, 10% enriched in the isotope 15N
$\delta\,^{15}N$	signature of ^{15}N in a sample when compared with a standard ($\delta\,^{15}N_{air} = 0$ ‰)
P	phosphate
P (d)	probability density function for leaf loss
PNUE	photosynthetic nitrogen utilisation efficiency
PPFD	photosynthetic photon flux density
Q_E	quantum efficiency
Q_o	irradiance at light compensation
Q_{sat}	irradiance at saturated Amax
R_d	calculated dark respiration
S	specific leaf area
S (d)	cumulative leaf survival function
SE	standard error of the mean
T	average daily temperature
T_b	base temperature below which leaf area growth ceases
VAM	vesicular-arbuscular mycorrhizae
Vc_{max}	maximum carboxylation rate of ribulose 1.5-bisphosphate carboxylase/
W	seed dry mass
W_d	plant dry matter
W_e	total dry mass of emerged leaves
W_l	leaf mass
W_s	total plant seed dry mass

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ABSTRACT

Assessing the impact of herbivory on plant growth and reproduction is important to predict the success of biocontrol of invasive plants. Leaf area production is most important, as photosynthesis provides the foundation for all plant growth and fitness. High levels of defoliation generally reduce the productivity of plants. However, leaf area production fluctuates during the season and compensational growth may occur, which both complicate accurate estimations of defoliation impacts. Under field conditions the interaction with neighbouring species and the availability of soil nutrients need to be assessed in order to gauge long term effects of weed invasions on natural environments.

In this thesis I have investigated seasonal leaf area dynamics in *Buddleia davidii* following repeated artificial defoliation, to quantify compensational leaf production and to understand the regulatory mechanisms involved. The impact of defoliation on photosynthesis, seed production, germination and nitrogen translocation patterns were analysed. Finally, possible facilitation between *B. davidii* and a native nitrogen fixer, *Coriaria arborea*, and the impact of *B. davidii* on soil nutrient availability were investigated.

In defoliated *B. davidii*, increased node production (34%), leaf size (35%) and leaf longevity (12%) resulted in 52% greater total emergent leaf area in the short term. However, with time and diminishing tissue resources the compensation declined. No upregulation of photosynthesis was observed in pre-existing leaves. Compensational leaf area production occurred at the expense of reproduction but the germination capacity of individual seeds was unaffected. In *B. davidii*, nitrogen reserves are stored in old leaves. Thus, the defoliation-induced decline in tissue reserves led to changes in the remobilisation pattern and increased the importance of soil uptake but biomass production especially that of roots had declined significantly (39%). Slight facilitation effects from the neighbouring nitrogen fixer and VA–

mycorrhizae were observed on *B. davidii* in the field, while its impact on soil chemistry during spring was negligible.

Defoliation of *B. davidii* resulted in priority allocation of resources to compensational leaf growth and a concomitant reduction in flower and seed production. The compensational leaf production greatly increased the demand for nitrogen, while continued leaf removal decreased the pool of stored nitrogen. This led to changes in nitrogen remobilisation and an increased importance of root uptake. However, the significant decline in root growth will likely impair adequate nutrient uptake from the soil, which is especially important where *B. davidii* invades nutrient poor habitats and will increase the success of biocontrol of the species. While mycorrhizae increase nutrient accessibility for *B. davidii*, it is likely that the additional stress of defoliation will negate the small facilitative effects from nitrogen-fixing species like *C. arborea.*

This research provides new insights into the mechanisms regulating leaf area dynamics at the shoot level and systemic physiological responses to defoliation in plants, such as nitrogen translocation. The compensation in leaf area production was considerable but only transitory and thus, the opportunity to alleviate effects of leaf loss though adjustment of light capture limited. However, to ascertain that photosynthesis at whole plant level does not increase after defoliation, more detailed measurements especially on new grown leaves are necessary. In general, defoliation had greatly reduced plant growth and performance so that an optimistic outlook for controlling this species can be given. Conclusions about the wider impacts of *B. davidii* on soil chemistry and community function will require further research.

Key words: *Buddleia davidii*, Defoliation, Nitrogen translocation, Species competition, VA-Mycorrhiza, Biocontrol, Leaf area, Seed production, Failure time analysis, Compensation, Photosynthesis

1

INTRODUCTION, LITERATURE REVIEW AND RATIONALE

1

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1.1 Introduction

1.1.1 Overview on the ecological significance and recent research of weed invasions

Unlimited human travel and trade throughout the world has resulted in overcoming the natural barriers of plant distributions (Luken and Thieret, 1997). Despite a growing awareness of possible negative effects of the uncontrolled introduction of non-native plants and an increased effort in many countries to prevent this, globally, increasing numbers of exotic invasive plants are becoming established in natural environments (Vitousek *et al.*, 1997).

These invaders pose a thread to natural environments. Here, they may compete for space and resources (Bellingham, 1998; D'Antonio *et al.*, 1998), displace indigenous flora (Tallent-Halsell and Walker, 2002; DeLoach *et al.*, 2003) which may lead to reduced species diversity (Standish *et al.*, 2001) and richness (Schooler *et al.*, 2003)and influence soil nutrients and change succession pathways with unknown long term effects (Wardle *et al.*, 1994; Rothstein *et al.*, 2004; Bellingham *et al.*, 2005). Invasive weeds may also cause significant loss in wood production in forest plantations as competitive understorey vegetation, unless expensive weed management is practised (Richardson *et al.*, 1993; Richardson *et al.*, 1999; Watt *et al.*, 2003b; Cathcart and Swanton, 2004).

Island flora seem to be especially vulnerable to invasive species. The proportion of exotic species is higher than in comparable mainland habitats (Lonsdale, 1999; Denslow, 2003).While it was first hypothesized that decreasing biodiversity increases invasibility of habitats (Elton, 1958; Mack *et al.*, 2000; Kennedy *et al.*, 2002), this is not always supported in nature (Lonsdale, 1999; Wardle, 2001). A recent review by Denslow (2003) proposes that the availability of resource and niches for invaders are more important to confer vulnerability to invasions onto habitats (Kitayama, 1996; D'Antonio, 1999). Island flora is generally less diverse and, for reasons of evolutional and geographical isolation, often misses taxonomic or functional groups or life traits (Simberloff, 1995).While invasive species experience unusual benefits from release of enemy pressure (Keane and Crawley, 2002), island natives seem to be less competitive than invaders (Cordell and Goldstein, 1999; Austin and Vitousek, 2000). Possible explanations include inbreeding depression, small population size, low seed banks (Drake, 1998) and inefficiency of dispersal (Carlquist, 1974). Furthermore, the number of endemics, which require special environment but have a small range size (Kelly, 1996), is higher (Eliasson, 1995). All these characteristics may create opportunities for invaders to fill in the gaps (Shea and Chesson, 2002), especially where propagule pressure is high (Rejmanek, 1989; Drake, 1998; Levine, 2000). Thus, special efforts must be made to battle weed invasions in islands (Dovey *et al.*, 2003). In New Zealand, great efforts are being made to maintain a high standard of biosecurity to ensure preservation and restoration of the unique indigenous flora (Towns, 1997).

Great interest exists in finding the reason for the success of invasive weeds. Invasive weeds are generally thought to have the characteristics of disturbance-adapted species with high growth capacity and fecundity (Denslow, 2003). However, such generalisations cannot be made, as species introduced by horticulture with a wide range of features (shade tolerance, vegetative spread) have the capacity to invade different habitats like forests

(Reichard and Hamilton, 1997). Daehler (2003) reviewed studies comparing invasive and native species and showed that while invasive species do not generally have a higher growth rate, fecundity or competitive ability when compared with coexisting natives, they did produce a greater leaf area and appeared to have lower tissue construction costs and a higher phenological plasticity. The latter was thought to be advantageous for growth in disturbed areas.

Invaders often experience a reduced pathogen pressure (damage from herbivores or fungal pathogens) in the new environment (Keane and Crawley, 2002). This was thought to allow alien invaders to be of taller stature and to produce larger seeds when compared to native species (Crawley *et al.*, 1996). Other advantages of alien invaders over natives included earlier flowering, longer-lived seed banks (Crawley *et al.*, 1996), a greater efficiency in capturing and utilising light (Durand and Goldstein, 2001) and a greater short term-resource use efficiency in nutrient poor environments (Funk and Vitousek, 2007). These characteristics may promote the successful establishment of the invaders because they allow them to access available resources and to occupy open niches more quickly than natives. Still, a comprehensive explanation of the success of invasions is not yet available.

In nature, plants are influenced in a number of ways by the environment (climate, soil substrate, elevation, latitude, microclimate) and neighbouring species (competition, facilitation), which makes the identification of a set of attributes that favour invasiveness very difficult (Binggeli, 1996). Woody invasive species, that invade natural areas do not have the attributes of the typical agricultural weed ("ideal weeds", as described by Baker (1965), are proposed to be mainly herbaceous, rapidly reproducing and abiotically dispersed species). Therefore, different management strategies than those aimed at agricultural weeds may be necessary to curb their spread (Daehler, 1998).

1.1.2 Management of invasive species

The most common method of weed control is the spraying of herbicides, but application can be difficult (Robinson *et al.*, 2000; Richardson *et al.*, 2004). Wind drift may cause an overdose or a sub-lethal dose and thus may not produce the desired impact on the weed, but may cause toxic effects on crop seedlings, indigenous fauna and flora (Peterson *et al.*, 1994; Kreuger, 1998) and human health (Sultatos, 1994; Calvert *et al.*, 2004). Widespread use of herbicide is no longer environmentally acceptable (McFadyen, 1998; Smith *et al.*, 2003) and mechanical removal is not feasible in remote areas. Both methods are costly and inefficient.

Biological control methods present an often successful, cost-efficient and safe alternative to the control methods mentioned above. This method involves the application of phytopathogens and insect agents - often from the same origin as the target plant, i.e. their natural enemies (Goolsby *et al.*, 2006). Biological control methods gain more acceptance (Delfosse, 2003) as host-specificity testing has become reliable (McFadyen, 1998) and the safety measures to date also include post-release evaluation of impact on the target and non-target plants (McNeill *et al.*, 2005). Biocontrol is self sustainable once the insects are established because their population will fluctuate with food availability (i.e. the target weed). Statistically, the United States, Australia, South Africa, Canada, and New Zealand use biocontrol approaches the most (McFadyen, 1998) but examples of successful weed control can also be found from Mauritius (*Opuntia vulgaris*, *O. tuna* and *Cordia curassaviaca* were completely suppressed (Fowler *et al.*, 2000a)) and the Pacific Islands (complete suppression of the two aquatic weeds *Eichhornia crassipes* Mitchell and *Salvinia molesta* (Mart.) Solms led to the restoration of a natural environment to its original state (Dovey *et al.*, 2003)). In Europe, especially the UK, application of biocontrol will likely be encouraged since the flexibility of this approach was successfully demonstrated against several invasive plant species including *Buddleia davidii* (Franch.) (Shaw and Reeder, 2003).

Biocontrol pathogens (fungi) or insects (leaf chewing or stem boring) generally reduce leaf biomass (Withers *et al.,* 2003; Li *et al.,* 2004) or inflict other damage on target plants (Hill *et al.,* 2000; Moran *et al.,* 2003) to reduce plant growth and fitness (Marquis, 1984; Davies *et al.,* 2003). Plants respond to herbivory either by resisting it (for instance through production of toxic compounds (Bennett and Wallsgrove, 1994; Zagrobelny *et al*. 2004)) or by tolerating it. Tolerance is principally achieved by compensatory growth of new leaf area. The relative costs and benefits of these responses to herbivory have been the subject of considerable ecological research (for recent reviews see Mauricio *et al.* (1997), Strauss and Agrawal (1999), Tiffin (2000) and Leimu and Koricheva (2006)). This research has usually been in the context of their effects on individual fitness, but when considered in a broader context, effects of herbivory and leaf loss on plant productivity and fitness can regulate community diversity and ecosystem productivity (Marquis, 2004). Therefore, research into plant responses to defoliation continues.

Artificial defoliation may not accurately reflect the effects of true herbivory, as the complex plant response is not triggered by tissue removal alone (Baldwin, 1990). This fact needs to be taken into consideration when interpreting the results of defoliation studies. Manual defoliation causes instant reductions in leaf area, while herbivory maybe a different stimulus because it is continuous. However, depending on the amount of insect damage, the leaf area reduction could be the same. Regardless, this method offers significant insight into the response of plants to leaf loss and is widely used in plant physiological studies, especially as a tool to evaluate bio-control agents prior to insect release.

1.1.3 *Buddleia davidii* Franch – an invasive shrub in New Zealand

Systematically, the species *Buddleia davidii* Franch. (buddleia; alternative spelling *Buddleja*) is placed in the family Buddleiaceae within the order Scrophulariales (Wagner *et al.*, 1999). It is a perennial shrub of Chinese origin, where it is native to Hupeh and Szechwan, the hilly regions of central and western China. It grows up to an altitude of 2700m and chiefly occurs on shingle banks of rivers (Miller, 1984).The average temperature in the native regions ranges 0-10 ºC in winter and 20-30 ºC in summer with an average annual precipitation of 1000-2000 mm (Hammond 1986 in (Starr *et al.*, 2003)). *B. davidii* tolerates a wide range of climatic conditions, including oceanic, continental and Mediterranean but prefers full sun and good drainage. It has no special requirements for nutrients, colonises a wide range of soils (chalk quarries, brick walls and disturbed areas in urban areas) and thrives vigorously on highly nutrient poor soils (Humphries *et al.*, 1982; Miller, 1984; Starr *et al.*, 2003). The only factors significantly influencing germination is the depth of seed burial (50% failure was observed at 0.5cm below surface), but a persistent seed bank is documented so that germination may commence after disturbance (Miller, 1984). The only restraint for the establishment of buddleia seedlings is the water supply. A significant drought resistance in seedlings develops only after four weeks, so that water shortage during the initial establishment phase can be critical (Miller, 1984).

B. davidii was, and still is (Wilson *et al.*, 2004; Phelan *et al.*, 2005) mostly introduced as an ornamental plant, but is now recognised as a major invasive weed in several countries. In Europe, it is listed as requiring high priority removal (Sheppard *et al.*, 2006) and can be found in Great Britain, France, Italy, Germany (Miller, 1984; Binggeli, 1998) and as far north as Norway (Invasive Species Specialist Group (ISSG) of the IUCN Species Survival Commission, 2007). A risk assessment of *B. davidii* for Hawaii and other Pacific Islands revealed the possible significant ecological or economic harm from buddleia

invasion (Binggeli, 1998; Starr *et al.*, 2003; Invasive Species Specialist Group (ISSG) of the IUCN Species Survival Commission, 2007) The species appears as a noxious weed on several watch lists in the United States (NWCB, 2007; TEPPC, 2007; USDA, 2007), is regarded as a potential environmental weed with a need for preventative control in Australia (Australian Government, 2007) and has become widely naturalised since 1946 in New Zealand (Webb *et al.*, 1988).

B. davidii invades open, disturbed habitats – it is found on roadsides, stream banks, new developments, abandoned areas, wastelands, pastures, open woodlands, and scree slopes (Binggeli, 1998). In these conditions it rapidly forms extensive monocultures (Miller, 1984). In New Zealand, it has become a dominant feature in primary successions in river beds, where it often coexists with a native pioneer species, the nitrogen fixing shrub *Coriaria arborea* Lindsey (Plate 1.1). Invading *B. davidii* may displace natural vegetation (Smale, 1990) and has been shown to compete strongly with trees in commercial plantations. In New Zealand, *B. davidii* infestations result in severe retardation in initial growth and establishment of *Pinus radiata* in forest plantations (Richardson *et al.*, 1996). The invasive success of *B. davidii* is attributable to its enormous growth potential. In river beds it recovers quickly after damage through resprouting, even after being submerged under up to 0.5*m* of substrate after flooding. The large numbers of wind dispersed seeds produced (the production of approximately 3 million seeds from an ‘average’ plant (Binggeli 1998 in Starr et al. (2003) may possibly be an annual figure) lead to quick reestablishment (Miller, 1984).Thus, once established, *B. davidii* is not easily removed.

Plate 1.1 Typical flood plain primary succession in the Hapuku river in the South Island of New Zealand. Dominating species are *Buddleia davidii* Franch. and *Coriaria arborea* Lindsey.

Controlling *B. davidii* invasion has proven to be very difficult. Common control methods are costly, inefficient and in remote areas often not feasible. Cutting induces resprouting, increasing plant density. The focus in New Zealand is now on biocontrol using insect herbivores as a safe and inexpensive alternative control measure to suppress *B. davidii* (Kay and Smale, 1990; Fowler *et al.*, 2003; Sheppard *et al.*, 2003; Sheppard *et al.*, 2006). Two insects, one leaf chewing and the other a stem boring insect, have been investigated as potential biocontrol agents since 1989. The Chinese weevil, *Cleopus japonicus* Wingelmueller, has proven to have the potential for reducing the rate of growth of *B. davidii* plants in experimental tests. Both larvae and adults graze the upper leaf surface, thereby causing the grazed leaf portion to dry out and die. If the area is large enough, leaf abscission may follow. The insects have been studied in a Forest Research containment facility in

Rotorua, New Zealand. *C. japonicus* produces 2-3 generations per season and over-wintering of eggs and pupae is crucial for the population build-up and dispersal. It may take several years to reach abundant insect levels (Kay *et al.*, 2003). Apart from the availability of food (buddleia leaves), the age of larvae and adults and the temperature is very important to determine the feeding activity of the insects (optimal feeding occurred at 21°C). Simulation studies on non-limited buddleia indicated that leaf area will be significantly reduced only from mid-summer to mid-winter, leaving the spring flush undamaged because here insect pressure may be too low *(Brockerhoff et al, 99).* More damage can be expected from larvae, which continue feeding as long as they stay on the plant, increasing the amount they consume with age, while it is not clear how long adults will stay on one particular plant (Withers *et al.*, 2003). In a high density treatment (20 larvae/plant) 83% of leaves showed leaf area grazing >50% (30% of the plants died) while in a low density treatment (3 larvae/plant) no mortality occurred. Grazing reduced stem height and total stem and branch length, dry weight of roots and shoots especially so in the high density treatment (Brockerhoff *et al.*, 1999). *C. japonicus* has been released in 2006 in New Zealand (ERMA, 2005) and appears to establish well (M. Watson, Ensis Forest Biosecurity and Protection, New Zealand, personal communication, June 2007).

1.2 Rationale of the present study

Previous research on defoliation impacts on *B. davidii* revealed the plant's ability to compensate for lost leaf area and presented the first information on the magnitude of defoliation needed to cause significant reduction in plant size (Watt *et al.*, 2004; Watt *et al.*, 2007). However, the complex interaction between insects and plants needs detailed

information on seasonal variations in the response of the plant host, as leaves provide the sustenance for the insect population and thus the foundation for the establishment of the control agent.

This project contributes to biocontrol management by providing data for modelling the seasonal leaf growth of *B. davidii* after defoliation. The data obtained will ultimately be used for linking seasonal leaf growth (the availability of food for *C. japonicus*) with the population dynamics of the insect agent. With a quantitative assessment of the impact of herbivory on plant growth and reproductive output in this invasive species the success of biocontrol management can be predicted. Furthermore, details of the defoliation impact on biomass production and nitrogen translocation in *B. davidii* are provided, which will allow greater understanding of the biological mechanisms involved in the recovery of the species after damage and thus may help predict long term effects of herbivory. An overview of the questions and objectives for this PhD thesis is illustrated in Figure 1.1.

1.2.1 Studying seasonal leaf area dynamics and its regulations

Establishment of the insect agent is a long term process and depends, apart from climatic conditions, on the availability of food (Kay *et al.*, 2003), which in this case is leaf tissue (see Fig.1.1 - 1). Seasonal leaf growth fluctuates naturally during the year. It is therefore necessary to evaluate the seasonal leaf area production (b) and quantify variability (i.e. the occurrence of new growth and natural loss on different shoot orders). Furthermore, defoliation may induce shifts in the seasonality of leaf area production (leaf area production may be prolonged or may commence earlier). So it is also vital to incorporate information about the temporal and quantitative responses of leaf growth when predicting the impact of leaf herbivores and thus the success of biocontrol (p) on the species (Withers *et al.*, 2003).

Apart from providing answers to these applied questions, the broader objectives of the thesis were to investigate the mechanisms (c) that regulate leaf area production and as such, compensational responses of plants to defoliation (d), which is of high general interest in plant physiology. Measurements of leaf phenology are very useful in providing data for detailed investigations of leaf size, leaf longevity, leaf growth rate and node production, which all contribute to an understanding of changes in crown architecture and thus the light capturing structures of plants after defoliation. Furthermore, an investigation of photosynthesis (a_i) is useful as defoliation-induced changes will affect the plant's resources of assimilates (a_{ii}) which form the foundation for all plant growth. This broadens our knowledge of the underpinning physiological mechanisms regulating systemic plant responses.

1.2.2 Trade–off between growth and reproduction

A current hypothesis assumes a trade-off between the cost for vegetative growth and the cost for reproduction in most plants (Obeso, 2002). Naturally, both aspects are important for a species' survival. Whether resource allocation after defoliation favours reproduction (e) or vegetative growth (b, f) seems to depend on the life form of species (Obeso, 1993). Where herbaceous species may compensate (Mabry and Wayne, 1997; Parra-Tabla *et al.*, 2004), woody species show less plasticity and generally reduced reproductive capacity in response to defoliation (Thalmann *et al.*, 2003; Mueller *et al.*, 2005). *B. davidii* has both a high growth potential and a massive reproductive output, which may facilitate quick reestablishment if germination is not impaired. Therefore, it is necessary not only to determine the effects of defoliation on plant growth (f) and leaf area production (b) but also on reproduction and seed germination (e). Linking data for flower and seed production with

leaf phenology and biomass data allows us to investigate the nature of the trade off between reproduction and growth in this species.

1.2.3 Nitrogen resources for growth

In addition to sufficient supplies of water, temperature and light, nitrogen (N) is often considered the most important limiting factor for plant growth (Binkley and Hart, 1989; Vitousek *et al.*, 2002b) (see Fig 1.1 - 2). It is essential for building amino acids and proteins, nucleic acids and secondary compounds (Larcher, 1995) but the highest concentration of N is found in leaves (Eckstein *et al.*, 1999) as it is present in components of the photosynthetic apparatus (a_i): proteins of the Calvin cycle and thylakoids (Evans, 1989). Nitrogen supplying growth is derived from soil uptake (g) or from internal storage (h). Stored nitrogen is particularly important for new growth or regrowth after damage where the soil supply may be insufficient (i.e. in spring or in N-poor environments). The tissue in which nitrogen is stored differs between species of different leaf habit and life forms. For example, deciduous species store nitrogen during the winter in woody tissues like roots (Tagliavini *et al.*, 1999), stems (Millard *et al.*, 2001) and bark (Cooke and Weih, 2005) while coniferous (Nambiar and Fife, 1987; Millard and Proe, 1993) and broad-leafed evergreen species (Wendler *et al.*, 1995; Cherbuy *et al.*, 2001; Millard *et al.*, 2001) store nitrogen in leaves. In species that store N in leaves, defoliation may deplete nitrogen reserves which may subsequently lead to changes in root uptake from the soil (i) and remobilisation (j), and may have implications for the photosynthetic capacity (a_i) and ultimate growth capacity (b, f) of the plants. Considering biocontrol with leaf herbivores, it is therefore important to determine the source tissue of nitrogen remobilisation (h) and the magnitude by which nitrogen cycling is affected by defoliation (j). Furthermore, in our general understanding of nitrogen translocation, a

question still remains as to where species with indeterminate leaf habit store nitrogen? These questions are addressed in the fourth chapter of this thesis.

1.2.4 Species interaction and impact on soil nutrient availability

In New Zealand, *B. davidii* mostly invades soils that are nutrient poor. In primary successions in river flood plains it quickly becomes a dominant feature (Bellingham *et al.*, 2005). Where ongoing defoliation slowly depletes the pool of stored nitrogen, it is hypothesised that soil uptake of N will increase in importance, but will the limited availability of soil N increase the vulnerability of the plants to defoliation?

It is necessary to view the invader not in isolation but as an integrated part of the natural environment (see Fig. 1.1 - 3). Here, the interaction with neighbouring species (k) is an important factor as establishment and growth of invading species are a function of many restricting (l) (competition for space, light and nutrients) and facilitating factors (m) (increased nutrient availability through N fixation and litter input or sheltering)(Callaway and Walker, 1997). In this context it is of great interest to investigate the influence that a nitrogen fixing neighbour has on an invader because the former can significantly increase soil nitrogen pools (g) (Vitousek *et al.*, 2002a; Walker *et al.*, 2003). In nutrient deprived habitats, this may likely increase growth and invasive spread of fast growing weed species like *B. davidii*. Furthermore, not only will neighbours influence invaders but the invaders will likely impact on the environment which it invades through introduction of novel functions (Levine *et al.*, 2003). Here, the impact on soil chemistry through litter deposition (n) may well be very important as soil nutrient availability is the foundation for establishment and composition of future plant communities. This project investigates possible facilitation and competition between the native nitrogen fixer *C. arborea* and the invasive *B. davidii* and the impact of *B. davidii* on soil nutrient availability in primary

successions in flood plains in New Zealand in order to estimate long term effects of weed invasion in the invaded natural environment.

Mycorrhizal associations (o) are very important in the plant –soil interface (Smith and Read, 1997). While they influence plant performance in many ways, an increased availability of nutrients (g) is the most important benefit to plants. The break down of otherwise inavailable sources of nitrogen and phosphorus by mycorrhizal fungi is especially important in nutrient deprived environments (Smith and Read, 1997; Read *et al.*, 2004) and can result in increased foliar nutrient concentrations (Tibbett and Sanders, 2002; Dickie *et al.*, 2007a). Despite low soil supply, unusually high foliar nitrogen and phosphorous concentrations are often reported for *B. davidii* (Matson, 1990; Bellingham *et al.*, 2005) which indicates that the species must have very efficient uptake mechanisms, such as possible facilitation from mycorrhizal association. However, the mycorrhizal status of *B. davidii* (o) remains unresolved to date, as the only two available reports (Stevenson, 1964; Harley and Harley, 1987) are contradictory. This study also aimed to resolve this question by investigating mycorrhizal infection in *B. davidii*.

To summarise, this project investigates the influence of artificial defoliation on photosynthesis in pre-existing leaves (a_i), seasonal leaf area dynamics (b), plant growth (f), reproduction (e) and nitrogen translocation patterns (h, i, j) and contributes valuable data to an ongoing biocontrol project against *B. davidii* Franch. in New Zealand. In order to integrate these general physiological questions into a broader ecological context of weed invasion in a natural environment in New Zealand, the possible facilitative effects (m) from a neighbouring nitrogen fixing species (*C. arborea*), the impact of *B. davidii* on soil nutrient availability (n) are also investigated. Furthermore, the mycorrhizal status of *B. davidii* (o) is clarified to elucidate a possible mechanism of high nutrient uptake capacity in this species.

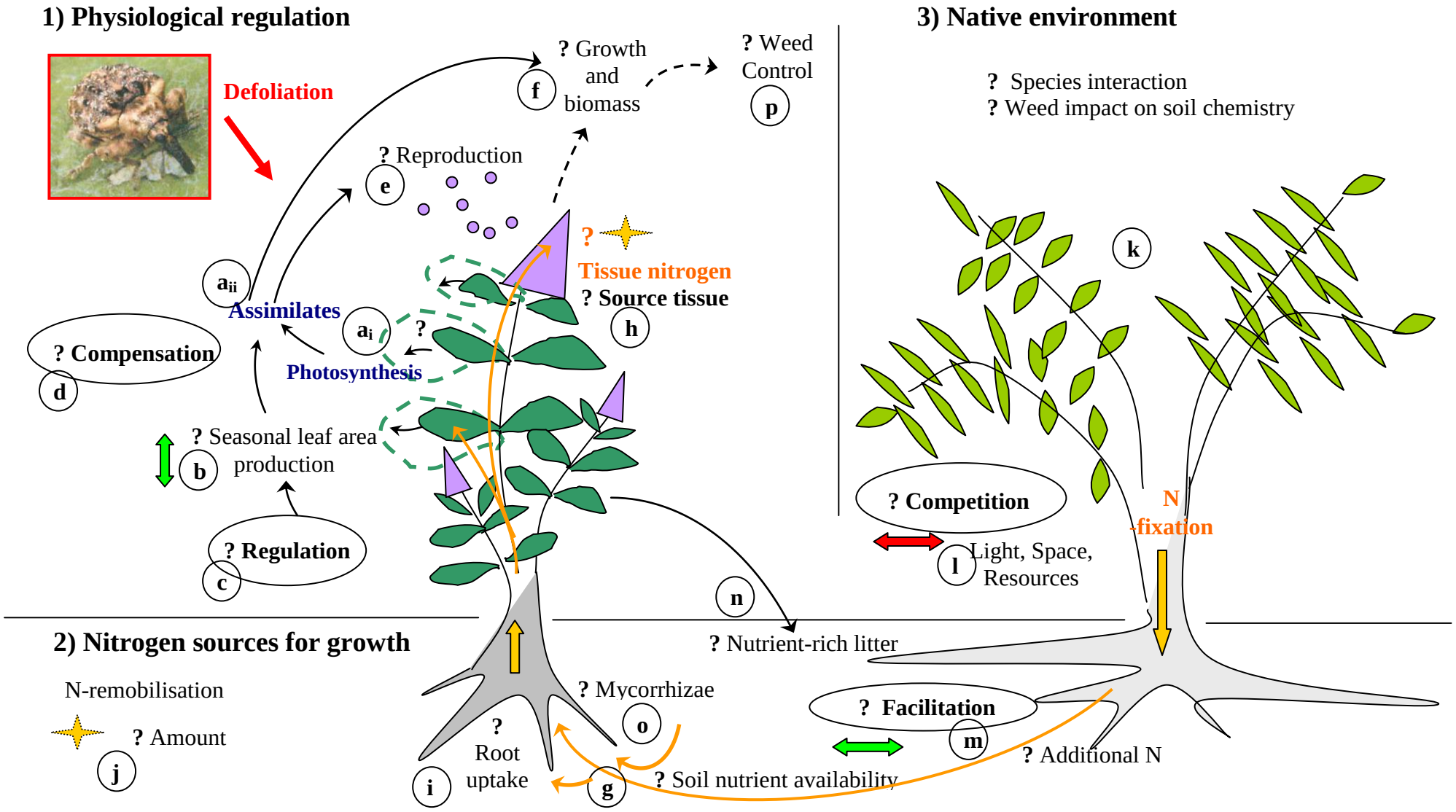

Figure 1.1 Questions and objectives for investigating the defoliation impacts on *B. davidii* Franch.

1.3 Overview of chapters

Chapter 2

A detailed description of the defoliation treatment, phenological measurements and leaf area calculations are given. The impact of defoliation on plant growth and seasonal leaf area dynamics is described to reveal high compensational leaf area production. Mechanisms (node growth, growth rate, leaf size, loss and longevity), which facilitated this compensation are explained. The different impact of defoliation on individual shoot orders are quantified and possible long term effects of the treatment are discussed. Mechanisms that facilitated the increase in light capture in defoliated plants are discussed in conjunction with the possibility of upregulation of leaf photosynthesis. (A modified version of the text has been accepted for publication to *Weed Research* in February 2008.)

Details of the measurements and results of photosynthesis in pre-existing leaves are given in an appendix at the end of the thesis.

Chapter 3

This experiment describes the defoliation impact on flower and seed production and on germination capacity of individual seeds. The trade off between reproduction and leaf growth in plants is discussed, highlighting that defoliation increases resource allocation to vegetative growth in this species (as described in Chapter 2), thereby decreasing reproductive output. (A modified version of the text has been submitted for publication to *Weed Research* in June 2007.)

Chapter 4

Here, the source tissue of nitrogen remobilisation in *B. davidii* is revealed and discussed in the context of growth habit in woody species. The defoliation induced reduction in biomass production and tissue resources of nitrogen are quantified and discussed in the context of high compensational growth (Chapter 2). Implications of the defoliation induced changes in nitrogen remobilisation and uptake for the success of biocontrol of *B. davidii* are explained and discussed in the context of soil nitrogen availability. (A modified version of the text has been submitted for publication to *Weed Research* in June 2007.)

Chapter 5

In order to view the results from the previous chapters in a broader ecological context, this chapter investigates species interaction effects between buddleia and a coexisting native nitrogen fixing species in the natural environment that the species invades. Growth, foliar nutrient concentration and soil nutrient availability are examined to reveal possible facilitative or competitive effects in both species. The impact of the invasive *B. davidii* on soil chemistry is discussed in the context of previous research.

Chapter 6

The importance of mycorrhizal fungi for nutrient uptake in plants is introduced. The mycorrhizal associations in *B. davidii* are investigated to explore a possible explanation for the high foliar nitrogen and phosphorus in buddleia, mentioned in Chapter 5.Other aspects of mycorrhizal associations with regard to weed invasions are discussed and areas of future

work highlighted. (**Dickie IA, Thomas MM, Bellingham PJ. 2007.** On the perils of mycorrhizal status lists: the case of *Buddleja davidii*. *Mycorrhiza **17:** 687-688.*)

Chapter 7

The final chapter presents a general discussion and conclusion from the results of the body of the thesis. Implications for the biocontrol management of this invasive species and areas of possible future research are discussed.

Appendix

Here, the investigations of the possible upregulation of photosynthesis in pre-existing leaves are described and discussed.

2

COMPENSATION IN SEASONAL LEAF AREA DYNAMICS AND LEAF LONGEVITY IN RESPONSE TO DEFOLIATION IN *BUDDLEIA DAVIDII* FRANCH

2

COMPENSATION IN SEASONAL LEAF AREA DYNAMICS AND LEAF LONGEVITY IN RESPONSE TO DEFOLIATION IN *BUDDLEIA DAVIDII* FRANCH

2.1 Introduction

Methods for controlling invasive plants often involve either the mechanical, chemical or biological reduction of biomass (Brockerhoff *et al.*, 1999; Dovey *et al.*, 2003; Kay *et al.*, 2003) or leaf area. Therefore, it is of considerable benefit to understand the efficacy of these approaches. However, there is also significant basic ecological interest in the responses of plants to leaf removal. Through their effects on plant productivity and fitness, herbivory and leaf loss can regulate community diversity and ecosystem productivity (Marquis, 2004).

Leaf removal generally reduces productivity in plants but mechanisms to compensate for the lost leaves have been demonstrated. These include increased photosynthetic rate in leaves (Hoogesteger and Karlsson, 1992; Vanderklein and Reich, 1999; Hart *et al.*, 2000; Thomson *et al.*, 2003; Retuerto *et al.*, 2004), specific leaf area (*S*), light use efficiency (Meyer, 1998; Watt *et al.*, 2007) and compensational leaf growth. Increased production of new leaves may occur through the release of apical dominance (Hjalten *et al.*, 1993; Strauss and Agrawal, 1999) or diverting resources to foliage production at the expense of reproductive output (Anten *et al.*, 2003). The trade off between reproduction and vegetative

growth is still unclear (after defoliation reproduction has been reported to increase (Mabry and Wayne, 1997) but also to decrease (Meyer, 1998; Newingham *et al.*, 2005)) and may depend on the growth form (Obeso, 1993). Leaf longevity is also important because increased longevity generally improves the cost efficiency of leaves. However, the response of leaf longevity to herbivory is very complex and requires further investigation (Chabot and Hicks, 1982; Ryser and Urbas, 2000) as here, also both decreased (Mabry and Wayne, 1997) and unchanged (Kudo, 1996; Watt *et al.*, 2007) leaf longevity has been observed. Because of the high variability in the response of these variables amongst species, no general rule can be inferred, so that to predict the effects of biocontrol herbivores more accurately, it is necessary to investigate these details in *B. davidii*.

Recent research aiming to predict the success of potential biocontrol insects on *B. davidii* and to determine the appropriate level of defoliation revealed a high compensatory leaf growth capacity in response to defoliation which can only be counteracted through repeated and severe defoliation (Watt *et al.*, 2004). The plants increased their canopy light capture (i.e. light use efficiency) mainly through increased biomass allocation to shoots for the growth of new leaves and increased specific leaf area (*S)* (Watt *et al.*, 2007). However, seasonal differences in the rates of leaf growth and loss, which are still unknown, may influence the impact of leaf removal on leaf area development. In order to understand the regulating mechanisms of this high compensational leaf area production, it is essential to quantify the seasonal impacts of defoliation on shoot growth and leaf characteristics (size, longevity and emergence).

This study investigated seasonal dynamics of leaf growth and loss in *B. davidii* for individual leaves within one year on control plants and plants which were manually defoliated to simulate herbivory. It was hypothesised that the effects of defoliation would depend on shoot age and order and that, when biomass allocation is directed to

secondary shoots, this would result in increased new leaf growth at the expense of older primary shoots where the rates of leaf growth would decrease. Further, it was hypothesised that, following partial defoliation, the size and longevity of remaining leaves would increase to compensate for the leaf loss.

2.2 Methodology

2.2.1 Experimental design and description of treatments

The experimental site was located at a nursery at Lincoln, New Zealand (latitude 43.6°S, longitude 172.5°E, elevation 12m above sea level). Meteorological data were obtained from Broadfield Station, approximately 1 km distant from the site. The daily mean temperature was 12.4°C with a range from 2.1 to 22.6°C. Total photosynthetically active irradiance for the year starting in summer was 6.7 GJ m^{-2}, with a daily maximum value of 33.2 MJ m^{-2} in early January 2005 (Fig. 2.1).

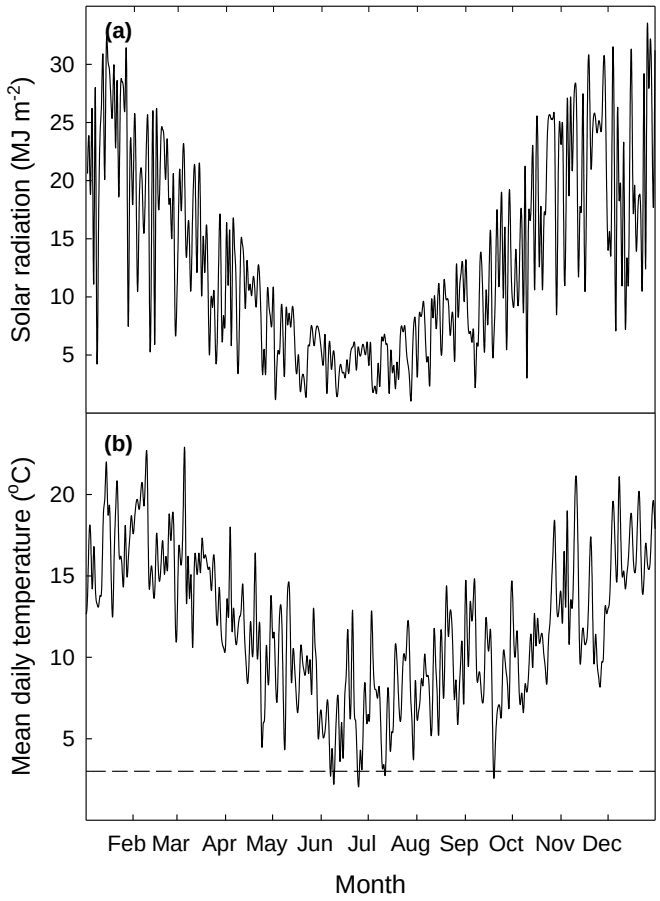

Figure 2.1 Seasonal changes in (a) total daily solar radiation and (b) mean daily temperature for the experimental site in 2005. The dashed line in (b) denotes the minimal growing temperature of 3°C for *B. davidii* that was used to calculate cumulative growing degree days

In midsummer (December 2004) 40 *B. davidii* plants, propagated from cuttings, where obtained from a nursery. Uniform seedlings (300 mm in height) were selected and transplanted into pre-washed fine sand in 30 L pots. The pots were then laid out in a randomised complete block design with a total of 20 blocks. Each block consisted of one defoliated and one control plant. The plants were irrigated weekly with a nutrient solution following Millard and Proe (1991) with a nitrogen concentration of 3 mM. For the defoliated plants, 66% of newly grown leaf area was removed manually by removing two of every three pairs of leaves (Fig. 2.2). The defoliation treatment was applied at four times in both growing seasons at monthly intervals from January to April (late summer) during the first season (17 January –14 June 2005) and from October to December (early summer) during the second season (28 June –28 December 2005) (Plate 2.1).

2.2.2 Plant harvest

In December 2004 six plants were harvested to obtain initial values of leaf dry mass, W_l. Five plants per treatment, representing average leaf area dimensions, were harvested at the end of the experiment to provide final values of W_l. All components were separated (leaves, sorted for each shoot type) and dried at 70^{o}C until constant mass was reached, then weighed. Subsamples of leaf area (one-sided basis) were measured at the start and the end of the experiment (December 2004 and 2005) using a leaf area meter (model LI 3100, Li-Cor Inc, Lincoln, NE, USA). The average (± 1 SE) specific leaf area, *S*, for both dates (28.4 ± 2.0 m^2 kg^{-1} when plants were young and 9.4 ± 0.4 m^2 kg^{-1} when plants were one year old) was determined from the quotient of area of fresh leaves and dry mass from the subsamples. Net leaf area for each plant, A_p, at the start and the end of the experiment was determined as the product of W_l and *S*. Seasonal changes in A_p for each plant were scaled from phenology measurements (described below).

Plate 2.1 Defoliation treatment. Buddleia seedlings grow very quickly (plants are **a)** two weeks (first defoliation), **b)** 2.5 months old) and **c)** in full flower at the age of 4.5 months).

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Table 2.1 Identification codes for shoot types on the measured branch, grown during the two seasons of the experiment in relation to the time of emergence and order as shown diagrammatically in Fig. 2.2

Shoot type	Season	Order
10	1	0
11	1	1
22	2	2
21a	2	1
20	2	0
21b	2	1

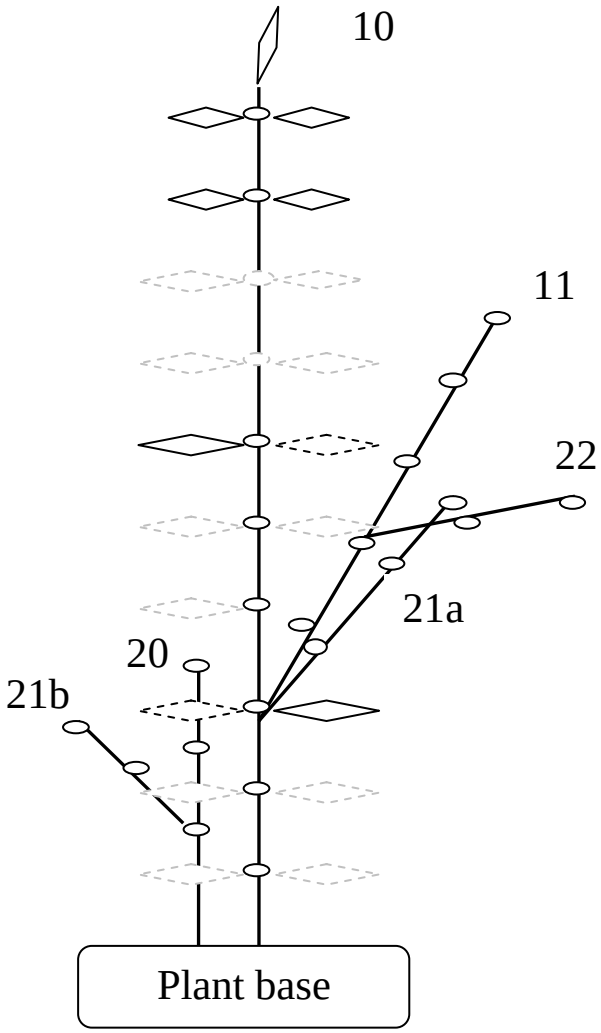


Figure 2.2 Shoot types on a sample branch over two seasons shown for the defoliated plants. Leaves, shown only for the main shoot (order 0), are present (black), naturally lost (black, dashed) and defoliated (grey). Each node grew two leaves and subsequently two new shoots of the same type. Identification of the codes is given in Table 2.1

2.2.3 Measurements of leaf phenology and plant growth

Phenological measurements of leaf area were made over the course of the growing season. On each plant a representative branch was selected, each node was numbered and the two leaves which occur on each node were classified as being either present, absent through defoliation or absent through natural causes. Side shoots that started growing on the branch were numbered with reference to the season of their emergence and their position on the main branch (Table 2.1, Fig. 2.2). On all nodes, with at least one leaf present, leaf width, L_w, and length, L_l, (mm) were recorded for a single leaf, and node leaf area A, (mm^2) was determined from these measurements using $A = 0.671\ L_l\ L_w\ n$, ($P < 0.001$, $r^2 = 0.97$, derived from a sub-sample of 120 leaves), where n is the number of leaves present at each node.

2.2.4 Scaling leaf area and leaf loss to branches

Phenological measurements of leaf area were used to determine branch level net leaf area (A_n) at the end of the growing season and cumulative emergent leaf area, A_e, over the course of the growing season. Leaf area of the main branch was determined from measurements, while the leaf area of side shoots was scaled to the branch level. Assuming each node produced two new shoots of the next order (Plate 2.2), the maximum node number on the parent shoot was multiplied with the measured leaf area on the side shoot. Total branch level leaf area was then determined by summing the total leaf area of the side shoots and the main shoot. Cumulative emergent leaf area was defined as the total leaf area produced over the growing season, which included all defoliated and naturally lost leaves over this period.

The rate of natural leaf area loss per plant was determined as the quotient of daily leaf area loss over the measurement period and net leaf area at the end of the period, with the natural leaf loss over the period included in the net leaf area estimate.

The average maximum leaf size for each leaf and shoot type and the proportional contribution of the leaf area from different shoot types to A_e were also calculated (Plate 2.2). Maximal node growth per plant and shoot type was used to calculate treatment differences in node increment per season.

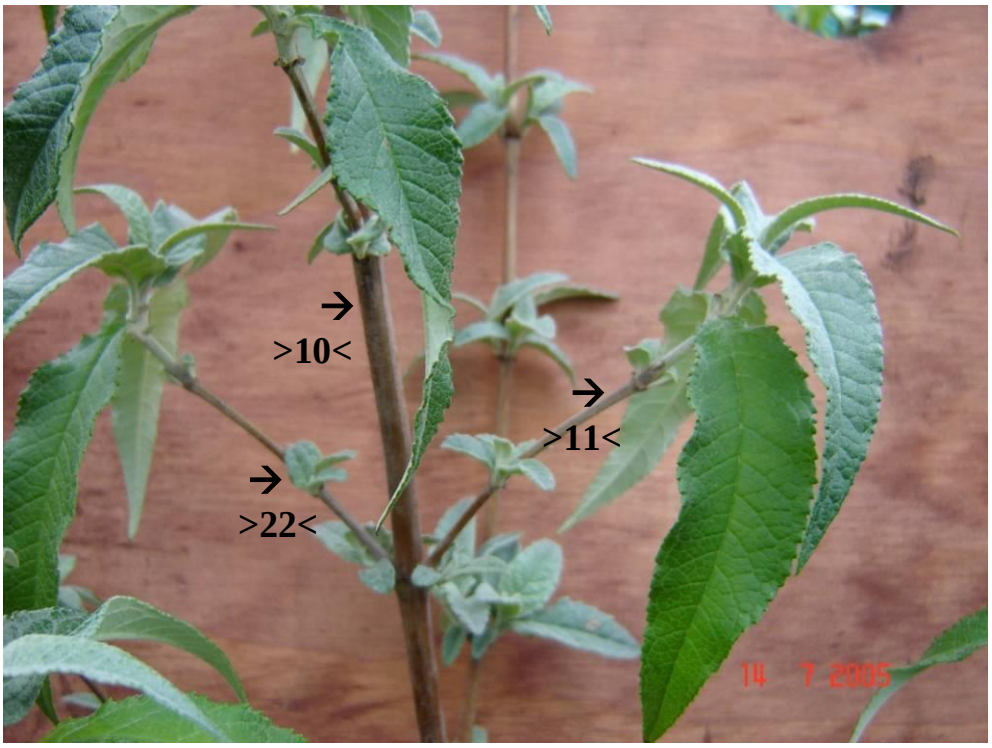


Plate 2.2 Differences in leaf size can be considerable between leaves of different shoot types. Shown is the main branch >10< (no leaves visible) in the centre, with well developed shoots no >11< (big leaves) and young shoots no >22< (small leaves) on a defoliated specimen of buddleia in the summer of the first growing season.

2.2.5 Calculations of leaf growth rate

Leaf area development in relation to temperature was calculated using A_e and cumulative growing degree days, G, calculated from

$$G = (T - T_b)\, d \qquad (1)$$

where T is the average daily temperature and T_b is the base temperature below which growth ceases and d the number of days. During the experiment, the base growth temperature for

B. davidii was estimated to be 3°C (no growth was observed below 2.9 ^{o}C in midwinter 2005 (19 May- 14 June)), as no leaf area increase was measurable at temperatures lower than this.

Cumulative leaf area was modelled in response to *G* using the von Bertalanffy equation (Richards, 1959) described as

$$A_e = a\,(1\text{-}e^{-b\,G})^{c} \qquad (2)$$

where *a*, *b* and *c* are empirical parameters. Differentiation of the von Bertalanffy equation allows calculations of maximum leaf area growth rate at the point of inflexion in the relationship, and the cumulative growing degree days taken to reach maximum growth rate, G_{max}. The cumulative growing degree days taken to reach 95% of the maximum leaf area, G_{95}, can also be calculated through rearrangement of the Eqn 2.

2.2.6 Statistical analysis

Data analysis was undertaken using SAS (SAS Institute, 1996). Variables were tested for normality and homogeneity of variance and transformations were made when necessary to perform the statistical tests. The influences of defoliation on cumulative emergence, net leaf area, mortality and plant dimensions were analysed using mixed effects model, with time as the repeated variable. Treatment differences in leaf area development per plant, node increment and leaf size were tested using general linear models. All multiple comparisons were undertaken using Tukey`s test.

2.2.7 Leaf longevity

Interval censored failure time analysis was used to examine patterns of leaf longevity, determined from phenological measurements. Definitions of minimum and maximum leaf lifespan were taken from Dungan *et al.* (2003). Failure time analysis allows estimation of probability functions describing age-specific leaf mortality risk. The probability density

function, $P(d)$, describes the probability that a leaf will die in the interval d to $d + \Delta d$ where d is the number of days. The survival function, $S(d)$, describes the probability that a leaf will live longer than d without dying.

The fit of six commonly used parametric distributions (normal, logistic, exponential, log-normal, Weibull and generalised gamma) to the survival time data from the two treatments (defoliation and undefoliated) and two growing seasons was tested. Of these distributions, the gamma (no logarithm) and Weibull distributions were selected for these four datasets as these had the maximum log likelihood, and a plot of the survival function against time showed that the model fitted the data well.

To investigate variation in leaf lifespan between treatments and growing seasons failure time analysis was used to determine the value of model parameters for each branch. A general linear model was then used to test if the main and interactive effects of defoliation intensity and growing season significantly influenced the value of these parameters.

2.3 Results

2.3.1 Plant dimensions

Defoliation of 66% of the leaf area reduced plant height by 10% with control plants reaching 1.48 m, and treatment plants only 1.34m at the end of the experiment (December 2005). The reduction of 19% in stem diameter growth was significant ($P < 0.01$) with values of 35.1 and 41.8 mm for defoliated and control plants, respectively (Plate 2.3). There was a significant (treatment × time) interaction for both diameter ($P = 0.03$) and height increment ($P < 0.001$).

Plate 2.3 Plant size of an undefoliated (left) and a defoliated (right) specimen of buddleia in March 2005. Plants are 4 months old.

2.3.2 Leaf area dynamics

Net leaf area development at the branch scale was calculated to reveal patterns of the response to leaf removal (Fig. 2.3). An instantaneous increase (average 22%) in leaf growth rate led to compensatory leaf area growth for defoliated plants compared with control plants (Fig. 2.3a). This effect in the second season was not as strong as that in the first season, so that continued defoliation resulted in final values for net leaf area in defoliated plants which were 12 and 17% lower at the end of the first and second seasons, compared with control plants (Fig. 2.3a). Thus, total net leaf area at the plant scale, $A_{p,}$ was similarly reduced in the treated plants by 21 (final values 0.87 m^2) and 25% (final value 1.15 m^2 $plant^{-1}$) at the end of the first and second seasons, respectively, compared to the control plants. Although the defoliated plants reached minimum leaf area 14 days earlier, leaf area maintained over winter (June –September) was 27% higher than that for the control plants, due to the earlier onset (by 42 days) of leaf growth in the second season shoot growth. The maximum increase in A_n attributable to the compensational growth in defoliated plants was observed in midwinter (July) with 67%.

Cumulative emergent leaf area, $A_{e,}$ clearly illustrates how defoliation alters leaf area production. At the end of the first season, total A_e was 52% higher in defoliated plants than the control (Fig. 2.3b). The maximum treatment difference in A_e was observed in midwinter (July) when A_e in defoliated plants exceeded that of control plants by 80%. This compensation effect was substantially reduced during the second season and the rate of leaf area production occurred at about the same rate as in undefoliated plants (Fig. 2.4b). As a result total emergent leaf area over both seasons in the defoliated treatment exceeded values for the control plants by 15% (Fig. 2.3b). The leaf area ratio A_e /W_d, calculated using the biomass values obtained from harvesting all plants in the second season, was significantly increased (by 45%) in defoliated plants (Table 2.2).

The daily rate of natural leaf loss from emergent leaf area was reduced in defoliated plants compared to control plants at all times except over winter (July – August), and mid summer of the second season (Fig. 2.3c). The defoliation-induced reduction in the rate of daily leaf loss averaged 37 and 21% during the first and second season, respectively. Significantly ($P < 0.05$) lower rates of leaf loss were observed in March (reduced by 88%) and May (reduced by 62%) in the first season (Plate 2.4).

Plate 2.4 Natural leaf loss in undefoliated (left) and defoliated (right) plants in Autumn of the first season (here April 2005). Plants are 4.5 months old.

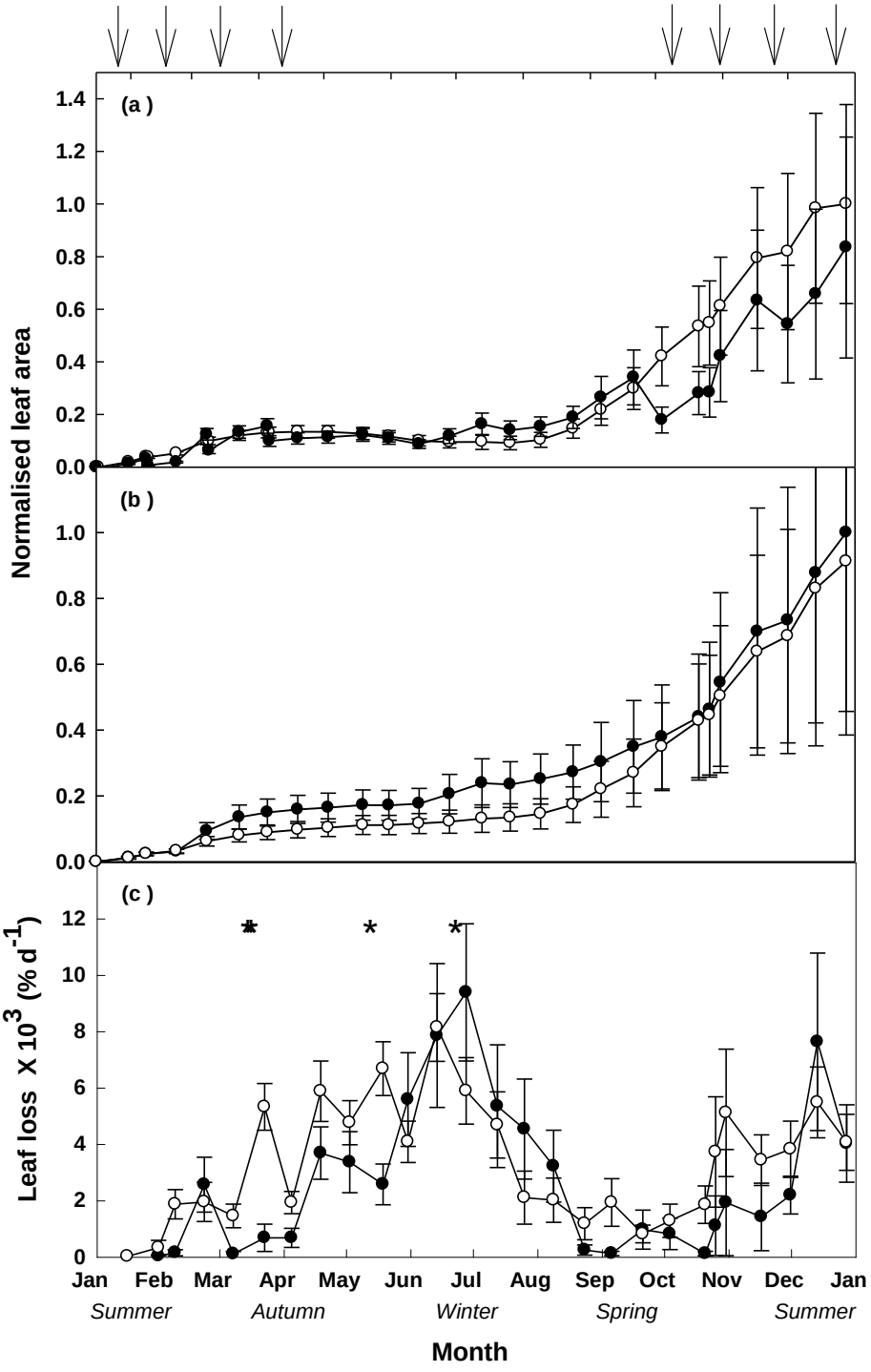


Figure 2.3 Normalised (a) net and (b) cumulative emergent leaf area production after defoliation shown as mean (± 1 SE) on the sampled branches for 20 defoliated (●) and 20 control (○) plants. Also shown is the rate of natural leaf loss (c) as mean (± 1 SE) percentage of remaining leaf area with significant differences * and ** at $P < 0.05$ and $P < 0.01$, respectively. The arrows indicate the times when defoliation was undertaken.

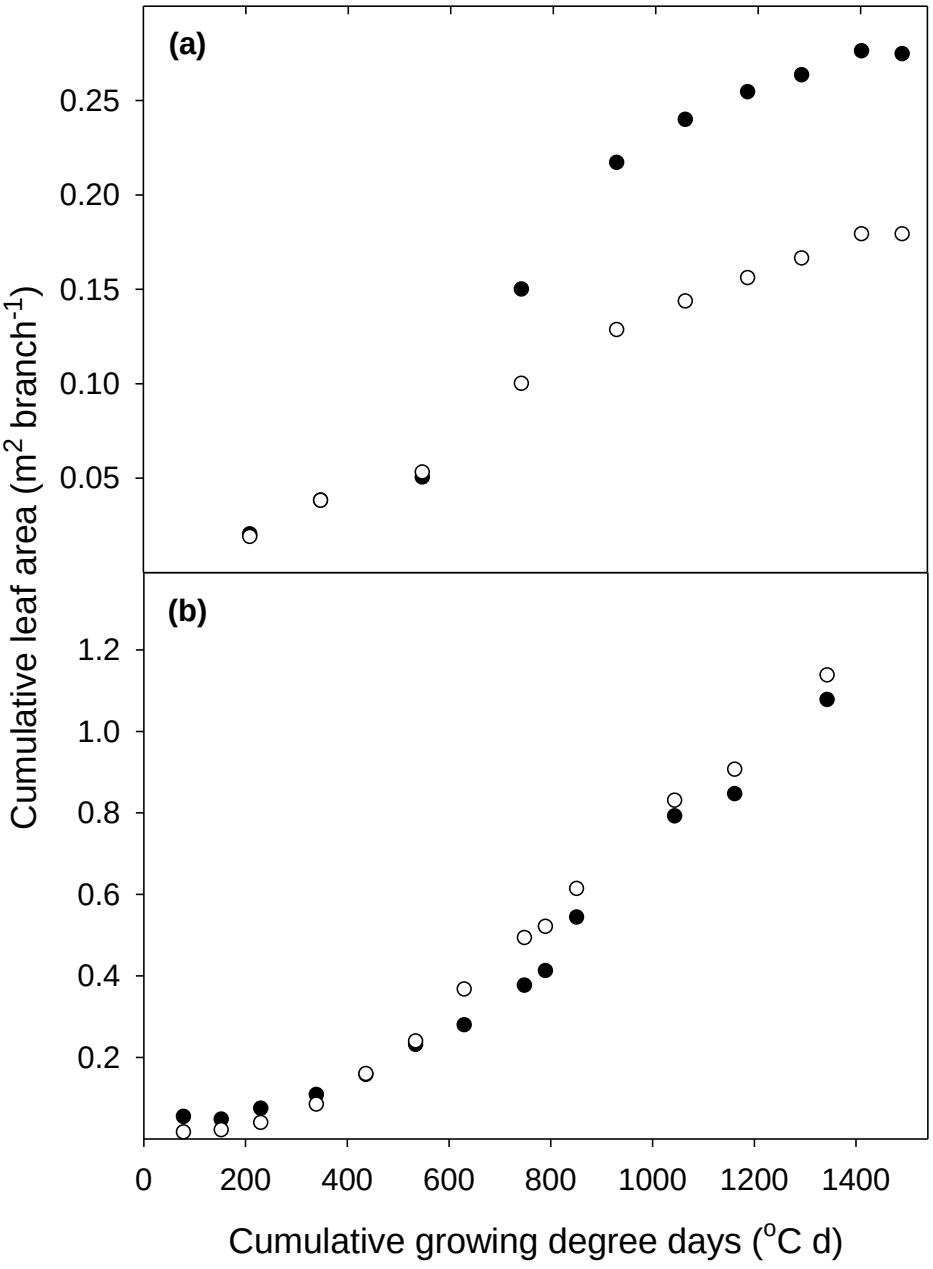


Figure 2.4 Relationship between growing degree days, *G*, and cumulative leaf growth during (a) first and (b) second seasons for the defoliated (●) and control (○) plants. Values shown are the averages of 20 plants. (Note: in (b) different initial values in both treatments were readjusted to zero, to be able to compare the two seasons with a different start date (16 January vs. 28 June))

Table 2.2 The impact of defoliation on the leaf area ratio in *B. davidii*. Shown are means ± 1 SE of the emergent leaf area (A_e) (n = 20), the total dry weight W_d (n = 5) and the ratio of A_e / W_d at the dates of harvest dates during the second growth season. A_e was derived from phenology measurements; W_d was obtained from destructive harvests in the second season, which is described in detail in Chapter 4. Differences between treatments were tested using a paired T-test.

Harvest date	A_e (m^2/ branch)		W_d (kg/ plant)		A_e / W_d	
	Defoliated	Control	Defoliated	Control	Defoliated	Control
26-Aug-05	0.45 ± 0.08	0.29 ± 0.05	0.18 ± 0.02	0.24 ± 0.02	2.44	1.20
27-Sep-05	0.57 ± 0.14	0.44 ± 0.1	0.19 ± 0.002	0.30 ± 0.01	3.05	1.46
18-Oct-05	0.72 ± 0.19	0.70 ± 0.17	0.25 ± 0.01	0.38 ± 0.02	2.92	1.84
7-Nov-05	1.13 ± 0.37	1.03 ± 0.3	0.28 ± 0.01	0.45 ± 0.03	4.02	2.29
4-Dec-05	1.19 ± 0.4	1.11 ± 0.32	0.37 ± 0.01	0.57 ± 0.04	3.23	1.96
6-Jan-06	1.61 ± 0.61	1.47 ± 0.46	0.46 ± 0.03	0.61 ± 0.03	3.51	2.42
T value	0.58		6.85		-12.2	
P value	>0.05		<0.002		<0.0001	
Dgf	8		5		5	

2.3.3 Response of leaf growth rate to cumulative temperature

The response of leaf growth rate to cumulative growing degree days modelled using the von Bertalanffy equation fitted the data well (r^2 = 0.99) (Fig. 2.4), revealing four major effects of defoliation. During the first season, total cumulative emergent leaf area increased 1.5 fold in defoliated plants compared with the controls (A_e = 0.28 m^2 versus 0.19 m^2) and the maximum growth rate was 1.9 times that of the control plants (3.2×10^{-4} *vs.* 1.7×10^{-4} m^2 [°C d]$^{-1}$). The cumulative growing degree days taken for leaf area growth to reach 95% of maximum value, G_{95}, was shortened by 35% in treatment plants (1629.2 versus 2500.0 °C

d), although the time taken to reach the maximum rate of leaf growth, was similar for both defoliated (526.9 °C d) and control plants (512.0 °C d) (Fig. 2.4a).

During the second season, the emergent leaf area increased by 1.272 m^2 *vs.* 1.268 m^2, so that the final values in total A_e were 1.61 *vs.* 1.47 m^2 in defoliated and control plants, respectively. The difference in the maximum rate of leaf growth between the treatments was negligible (Fig. 2.4b) within the range of the data (1.23×10^{-3} m^2 [°C d^{-1}] defoliated plants *vs.* 1.20×10^{-3} m^2 [°C d^{-1}]), but the cumulative growing degree days to reach maximum growth rate was 37% longer for the defoliated plants (1105.1 °C d) compared with the control plants (805.6 °C d).

2.3.4 Contribution of shoot types, leaf size and node growth to emergence leaf area

For the control plants in the first season, an average of 40% of the total leaf area occurred in the primary shoots (type 10), with the balance (60%) in first order shoots (type 11, Table 2.3). Although there were six shoot types present during the second season, the leaf area was mainly on type 11 shoots (28%) and type 22 shoots (57%). In both seasons, the proportional contribution of secondary shoots to total emergent leaf area was greater in defoliated plants than control plants. This effect was most pronounced in the most active shoot for the season, i.e., first order (type 11) shoots in the first season (72%) and second order (type 22) shoots in the second season (64%).

Table 2.3 Percentage contribution of different shoot types (Fig. 2.2) to total cumulative emergence leaf area , A_e, at the end of each season for defoliated and control plants.

	First season				Second season			
	Defoliated		Control		Defoliated		Control	
Shoot	A_e (m^2)	%	A_e (m^2)	%	A_e (m^2)	%	A_e (m^2)	%
10	0.083	28.1	0.078	40.3	0.080	4.7	0.077	5.2
11	0.212	71.9	0.116	59.7	0.412	24.0	0.413	27.7
22					1.093	63.6	0.871	58.4
21a					0.080	4.7	0.095	6.4
20					0.027	1.6	0.026	1.8
21b					0.026	1.5	0.009	0.6
Total	0.294	100	0.194	100	1.719	100	1.490	100

The defoliation induced shift in leaf area distribution among shoot types was mirrored in the pattern of node production (Table 2.4).

Table 2.4 Mean (± 1 SE) increase in node numbers for defoliated plants compared with control plants for both growing seasons. Treatment differences were tested using ANOVA and the symbols * and ** represent significant differences at $P < 0.05$ and $P < 0.01$, respectively.

	First season			Second season		
Shoot	Defoliated	Control	Increase %	Defoliated	Control	Increase %
10	9.7 ± 0.3	8.9 ± 0.3	8.8	0.1 ± 0.1	0.0	100.0
11	147.2 ± 2.7	109.7 ± 0.9	34.2**	140.9 ± 27.2	137.3 ± 22.7	2.6
22				1446.1 ± 546.8	777.6 ± 331.3	86.0*
21a				113.7 ± 32.9	63.3 ± 28.1	79.6
20				12.3 ± 1.6	10.6 ± 1.5	15.7
21b				36.1 ± 15.6	12.3 ± 8.2	194.6

While all shoots in the defoliated plants displayed an increase in the rate of node development in comparison to the control, the largest defoliation induced gains were observed in first order (type 11) shoots in the first season 34% ($P < 0.01$) and second order (type 22) shoots in the second season 86% ($P = 0.02$).

Table 2.5 Mean (± 1 SE) maximum leaf size (mm^2) and the percentage increase in leaf size for the defoliated plants compared with the control plants for both growing seasons.

	First season			Second season		
Shoot	Treatment	Control	Increase %	Treatment	Control	Increase %
10	3216.3 ± 134.6	3367.9 ± 127.2	- 4.5	1795.9 ± 429.6	1378.7 ± 157.0	30.3
11	777.1 ± 66.9	573.5 ± 54.2	35.5	688.8 ± 54.5	878.1 ± 50.0	-21.6
22				472.3 ± 125.4	628.9 ± 79.4	-24.9
21a				355.2 ± 50.7	739.5 ± 122.8	-52.0
20				1209.3 ± 105.7	1385.7 ± 112.3	-12.7
21b				359.2 ± 84.6	341.4 ± 175.3	5.2

Defoliation induced gains in maximum leaf size were of a similar magnitude to gains in node production during the first season for first order (type 11) shoots (35%, $P < 0.05$, Table 2.5). However, during the second season, maximum leaf size in first (type 11) and second (type 22) order of defoliated plants was reduced by 22% and 25% compared to control plants.

2.3.5 Leaf longevity

Defoliation prolonged leaf longevity in all cohorts (Fig. 2.5). The cumulative survival function *S* (*d*) illustrates that the 50th percentile of leaves in defoliated plants survived 13 days longer (108 versus 121 days) during the first season and 21 days longer (89.1 versus 110 days) in the second season compared with leaves on the control plants (Fig. 2.5a). Thus, defoliation increased overall leaf longevity in treatment plants by 12% in the first season *(P* < 0.05) and by 23% in the second season *(P* < 0.001). From the probability density function, *P* (*d*), the highest rate of mortality after emergence occurred 13.3 days later in defoliated plants (90 *vs*. 103 days) in the first season and 19 days later (86 *vs*. 105 days) in the second season, compared with control plants (Fig. 2.5b). The greatest treatment difference of 31 days (38%) (*P* < 0.001) was found in leaves grown on new shoots in the second season, when leaf life span was 111 and 81 d for the defoliated and control plants, respectively. This suggested that defoliation-induced enhancement of leaf longevity increased with time, supporting the results of reduced leaf loss in defoliated plants (Fig. 2.3c).

In both treatments, leaves growing in the second season were generally shorter-lived than those growing in the first season. However, in the second season, leaves growing on first season shoots (types 10 and 11) survived much longer (*P* < 0.01) than all other leaves (Fig. 2.5). The 50th percentile of those leaves survived 183 and 165 d on defoliated and control plants, with a treatment difference of 18.6 days (11%). Compared with values in the first season, longevities of these leaf types were 62 and 57% greater for defoliated and control plants, respectively. Comparing values with those of all other leaves grown in the second season, the difference in leaf longevity amounted to 64% and 104% for defoliated and control plants, respectively. This is an important result as leaves on shoot types 10 and 11 contributed more than a third of the emergent leaf area in both treatments (Table 2.3).

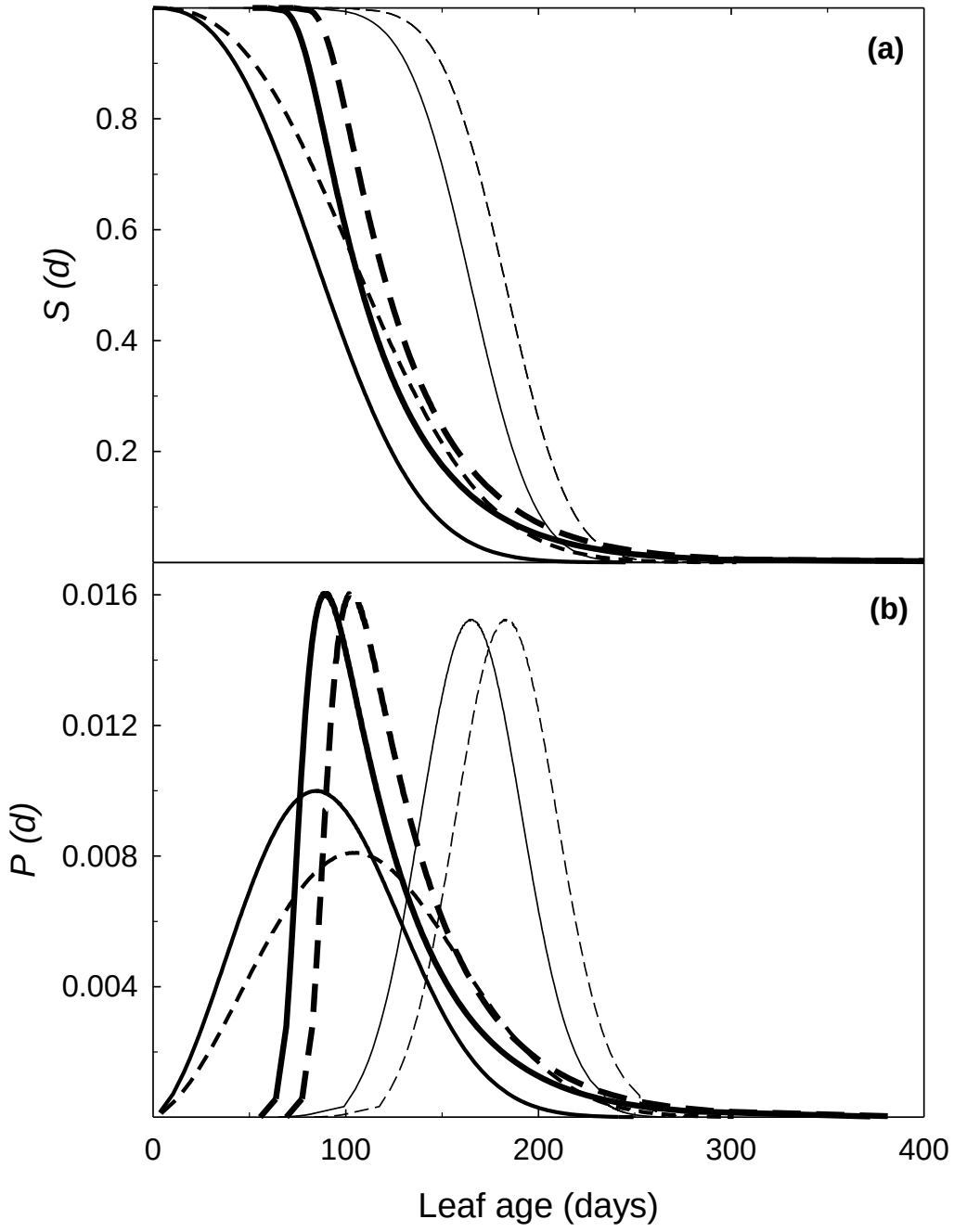


Figure 2.5 (a) Survival function *S (d)*, showing the probability of leaves surviving to a given time *d* and (b) Probability function *P (d)* showing the probability of leaves dying at a given time *d* after emergence for the treated (dashed lines) and control (solid lines) plants. The thickness of the lines refer to leaves of the first season (bold), second season (medium) and second season leaves grown on first season shoots (thin), derived from data of 20 plants (all shoot types pooled) per treatment

2.4 Discussion

This study clearly demonstrates that while *B. davidii* showed a high plasticity in compensation to defoliation, the treatment impacted greatly on seasonal leaf area production and general plant growth. Here, time is of special importance. The species showed a remarkable regrowth capacity following defoliation during the first season, with emergent leaf area in defoliated plants exceeding that of the control by 52%. This response resulted from higher node production, increased leaf size and increased leaf longevity. During the second season, the compensation in defoliated plants was much reduced as a defoliation-induced reduction in leaf size completely negated the effects of increased node production and leaf longevity. Although the total leaf area production at the end of the experiment in defoliated plants still exceeded that of the control by 15%, the vigour of the compensatory response was considerably reduced after just two growing seasons (Plate 2.5). The increase in canopy light capture through adjustments of leaf characteristics was the main measure to increase potential carbon assimilation (no evidence of upregulation of photosynthesis in pre-existing leaves was seen; see the Appendix for details) and was outweighed by effects of the continued leaf removal. This may indicate that, with ongoing defoliation, compensation may cease completely and the impact of defoliation will increase with time.

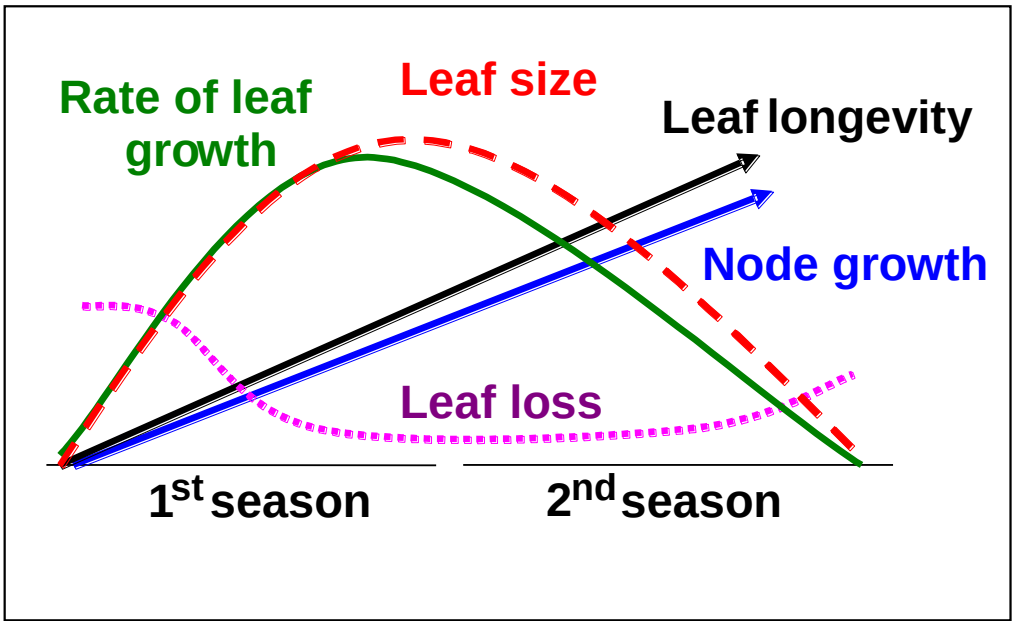


Plate 2.5 The mechanisms involved in the compensation for defoliation in *B. davidii*. After a strong response in the first growing season, the vigour of the compensation was much reduced.

2.4.1 Compensational responses and regulation of leaf area dynamics

2.4.1.1 Canopy light capture and resource allocation

This research clearly shows that *B. davidii* increases canopy light interception in response to defoliation. The leaf area ratio A_e/W_d (emergent leaf area/ plant dry matter) increased by 46% in comparison to undefoliated plants (Table 2.2) through increased regrowth of foliage (facilitated by increased node growth and early bud break), increased leaf size and longevity and reduced leaf loss. The increase in canopy light capture in response to defoliation is supported by Watt *et al.* (2007), who observed increases in light use efficiency (14%) in 66% defoliated *B. davidii*. During winter, a 27% higher leaf area was maintained in defoliated buddleia plants, which was attributable to an earlier (42 days) initiation of leaf growth in buds on new season shoots. This would indicate an increased demand for photosynthates (and also provides useful information about the availability of leaf material as food source for the insect population). However, the extent to which adjustments in leaf, and hence canopy, properties (i.e. leaf size, longevity or specific leaf area) can successfully

alleviate the negative effects of leaf area reduction may be limited, especially in the light of declining resources. A significant decline in the size of individual leaves indicated a reduced vigour of compensation during the second season. Node development and leaf longevity were the only mechanisms that still increased in defoliated plants but this did not lead to higher rates in leaf area growth in the second season.

Most studies report a decline in general plant growth and performance after repeated defoliation, despite transitory increments in photosynthesis and light capture (Nykanen and Koricheva, 2004). Defoliated *B. davidii* have been shown to facilitate the regrowth of leaf area with much greater daily biomass allocation to leaf growth (41%) (Watt *et al.*, 2007). Such shifts in resource allocation (also reported for trees and herbs (Mabry and Wayne, 1997; Lavigne *et al.*, 2001)) may be induced by an increased sink strength of growing meristems (Whigham, 1990) and may be regulated by re-partitioning and remobilisation of nutrients within plant tissues (Lemaire and Millard, 1999). High compensational leaf growth demands additional resources and must therefore draw largely from stored reserves, especially N (Millard and Proe, 1991; Stephens *et al.*, 2001; Carswell *et al.*, 2003; Lattanzi *et al.*, 2005). Thus, the significant reductions in stem diameter and height growth observed in defoliated plants in this study suggest limitations to growth from declining sources of carbon and nitrogen. This is further supported by the decline in the vigour of the compensational response during the second season which was concomitant with significant reductions in biomass (observed also in *Centaurea uniflora* (Newingham *et al.*, 2005)) and tissue N reserves (Chapter 4) and highlights the fact that continued high-level defoliation is most important to increase the impact of defoliation on leaf production and plant productivity.

2.4.1.2 Leaf longevity

Relationships between leaf longevity and physiological characteristics of leaves are very complex and often still inconclusive (Chabot and Hicks, 1982). Enhanced leaf longevity prolongs the time for photosynthesis and also increases the cost efficiency of leaves (i.e. reduces the relative carbon cost for leaf production in long lived leaves) (Chabot and Hicks, 1982). Here, leaf longevity was positively correlated with the cost of leaf construction per unit carbon gain (Williams *et al.*, 1989; Cordell *et al.*, 2001). Leaf life time can also be a major determinant of nutrient use efficiency in plants because of a prolonged retention of nutrients in long lived leaves (Escudero *et al.*, 1992, Eckstein *et al.*, 1999; Diemer, 1998). Fertilisation with nitrogen reduced both leaf longevity and nitrogen use efficiency (Cordell *et al.*, 2001). Further determinants for the longevity of leaves may include the emergence time (Dungan *et al.*, 2003) and environmental parameters, such as low nutrient availability where preservation of nutrients is important (Eckstein *et al.*, 1999).Very few data exists for the relationship between leaf longevity and defoliation (Chabot and Hicks, 1982). Remaining leaves on defoliated plants may be expected to increase in longevity, as the reduction in photosynthetic capacity from leaf loss would increase the value of the remaining leaves. However, some species responded to defoliation with decreased leaf longevity (Mabry and Wayne, 1997) while in other species leaf longevity increased (Meyer, 1998) or did not change (Kudo, 1996). In *B. davidii*, defoliation significantly increased leaf longevity and the treatment difference increased with time.

Failure time analysis provided a useful means for determining not only the treatment difference in leaf longevity over time, which was not evident from examination of temporal fluctuations in leaf loss, but it also revealed that leaves from different shoot orders and ages varied widely in longevity. In both treatments, leaves on old shoots lived longer than those on the ontogenetically younger side shoots. In *Aristotelia serrata* the longevity of leaves on

main shoots (older) was also higher than that of leaves on side shoots (younger) (Dungan *et al.*, 2003), but the physiological mechanisms are not yet understood. Interestingly, new leaves grown on previous-season shoots (i.e. regrowth on old main shoots) lived much longer than all other leaves. This pattern was observed in both treatments, regardless of the treatment difference between defoliated and undefoliated buddleia plants (Fig. 2.5).

It may be hypothesised, that leaf longevity may be higher while plants are young and have only few leaves and that when plants grow and their leaf number and turnover rate increases leaf longevity may decline. While this would explain the generally shorter life span of leaves grown in the second season in comparison to those grown in the first season, it does not explain why leaves regrown on previous season shoots lived so much longer. Since this occurred in both treatments this was not a defoliation effect. The position of shoots (according to their emergence time) within the canopy also determines their light environment which then influences shoot growth (Suzuki, 2002; Suzuki, 2003). Therefore light availability should also influence leaf longevity. If this was true one would expect to see greater divergence in leaf longevity between leaf cohorts on undefoliated and defoliated plants (where the light environment would have changed markedly due to leaf clipping) but this was not obvious and would require further study. A further possibility is longevity of leaves is influence by their position on the shoot which reflects their distance from sinks (for instance, flowers at the end of shoots or meristems on shoot tips). Here, leaves that are closer to important sinks may increase in longevity because they may act as a major source of assimilates. However these hypotheses would require further and more detailed investigations.

2.4.1.3 Leaf phenology for individual shoots

Few studies have investigated the impacts of defoliation on branching pattern and whether these effects translate into differences in productivity. The onset of the defoliation impact was clearly reflected in leaf size (Table 2.5). An increase in leaf size in response to defoliation was already noted on the first main shoot (the first shoot produced early in the first season). During the second season, leaf size on almost all shoot types on defoliated plants was reduced and almost completely negated the effects of increased node production and leaf longevity. Leaf size may be regulated partially by hormonal controls (Haukioja *et al.*, 1990), but here limitations in the nitrogen and assimilate supply to leaf growth is likely to be the major factor (see Chapter 4 for details on nitrogen translocation). A similar observation was reported for *Picea glauca*, where the reduction in leaf size was directly related to the amount of herbivory (Quiring and McKinnon, 1999) and in *Eucalyptus nitens*, where both leaf size and total leaf area increased after 50% pruning, but decreased after 70% pruning (Pinkard and Beadle, 1998).

How much leaf size was reduced amongst shoot types may reflect the proportion of assimilates supplied to shoots of different emergence time. In buddleia, the greatest reduction in leaf size was observed on the leaves on the youngest side shoot of the old main shoot in the inner canopy (shoot type 21a), while leaves on the new main shoot and its first order shoots (ontogenetic youngest shoots also in the inner canopy; shoot types 20, 21b), showed the least reduction in leaf size in response to defoliation. It would seem that leaves on those youngest shoots still received the greater share of the (reduced) resources and showed the smallest proportional size reduction amongst all shoots in defoliated plants.

It is interesting that, in buddleia, the main shoots bore the largest leaves, regardless of the shoot age, while the leaf cohort that contributed most to the emergence leaf area production at plant level (shoot type 22 with more than half of the total leaf area) had only

medium sized leaves. These shoots also responded with the highest increment in node production after defoliation. Their major contribution to the total plant leaf area is therefore mainly attributable to their great number of nodes and thus leaf numbers and not size of individual leaves. This would suggest that node development is more important for leaf area production than leaf size. The success of biocontrol could be improved by introducing a second insect that attacked shoot tips to stop shoot elongation.

2.5 Summary

Defoliation caused strong short term compensation in leaf area growth. This response was facilitated by increased allocation of resources to leaf growth and was mainly achieved through increases in leaf size, accelerated node production and increased longevity of individual leaves. In the second season, compensation markedly weakened, which was mainly due to a significant decline in leaf size. Further increased leaf longevity and node production did not compensate for the smaller leaf size. A temporal shift in leaf phenology (early bud break and new leaf growth in spring in the defoliated treatment) also indicated an increased demand for assimilates. The quantitative analysis of the patterns of leaf growth and death contributes to our understanding of the regulation of compensational leaf growth at shoot level and provides the basis for modelling the impacts of inter-annual changes in leaf area on productivity. The results suggest that the impact of defoliation on the overall fitness of invasive species with indeterminate growth may be less significant in the short-term than would be predicted, due to compensation, but that this may increase strongly over time if defoliation continues.

3

INFLUENCE OF DEFOLIATION ON REPRODUCTIVE CAPACITY AND SEED GERMINATION IN *BUDDLEIA DAVIDII*

3

INFLUENCE OF DEFOLIATION ON REPRODUCTIVE CAPACITY AND SEED GERMINATION IN *BUDDLEIA DAVIDII*

3.1 Introduction

Annual weeds and primary colonising species that are able to survive and establish rapidly in disturbed habitats are usually characterised by high seed production and fecundity (MacArthur and Wilson, 1967; Sakai *et al.*, 2001). To reduce the invasive potential of weed species, it is necessary to reduce both vegetative growth and reproductive potential. As the mass of individual seed is often positively correlated with seedling survival and vigour (Armstrong and Westoby, 1993) the success of weed control is likely to be maximised by reducing both the number and mass of seeds (Hunt, 2001; Mueller *et al.*, 2005). Despite high variability in the number of flowers, *B. davidii* produces large amounts of wind dispersed seeds that can reach up to 2 to 3 million seeds per year for a typical adult plant (Miller, 1984), assisting the rapid establishment of new invasive plants on disturbed sites.

Defoliation is known to induce compensational responses in plants. In the short term, increased photosynthesis and longevity of remaining leaves (Meyer, 1998) and the redistribution of resources to promote new leaf growth (Hoogesteger and Karlsson, 1992; Anten *et al.*, 2003) may occur to compensate for the lost photosynthetic tissue (Anten *et al.*,

2003; Parra-Tabla *et al.*, 2004; Chapter 2). While the timing and degree of leaf removal (Lee and Bazzaz, 1982; Marquis, 1992) are known to impact on seed and fruit production (Stephenson, 1981; Pinkard and Beadle, 1998; Tong *et al.*, 2003), less is known about the competitive trade off for resources between reproduction and vegetative growth (Obeso, 2002) and their relationship with phenology and nutrient availability (Obeso, 1993). In woody perennials, biomass and reproductive capacity generally decline with leaf removal (Hoogesteger and Karlsson, 1992; Vanderklein and Reich, 1999; Thalmann *et al.*, 2003; Mueller *et al.*, 2005), whereas the response in herbaceous species varies widely (Parra-Tabla *et al.*, 2004; Newingham *et al.*, 2005). The herbaceous plant *Cnidoscolus aconitifolius* (Euphorbiaceae) was shown to fully compensate in seed mass after defoliation (Parra-Tabla *et al.*, 2004) and for the annual herb *Abutilon theophrasti* an increase in reproduction capacity in response to defoliation has been reported (Mabry and Wayne, 1997). This compensation may be facilitated by a higher plasticity in herbal species compared to woody species (Obeso, 1993).Despite *B. davidii* being a woody perennial, its success in colonising open and disturbed places may in part be attributable to very high seed production, growth capacity and fecundity typical of herbaceous plants.

Investigations of seasonal leaf area dynamics in response to 66 %defoliation of new leaf area in *B. davidii* (Chapter 2; Watt *et al.*, 2007) revealed an increase in leaf area production up to 50 %. This study reports the impacts of repeated defoliation on seed production and germination in relation to leaf area dynamics in this species. *B. davidii* combines attributes of herbal perennials (high fecundity and growth plasticity) with a woody physiognomy. It is hypothesised that, with limited photosynthetic resources following defoliation, the ability to compensate for leaf loss will be at the expense of seed production.

3.2 Methodology

3.2.1 Experimental sites and seed source

Eighty *B. davidii* seedlings, propagated from cuttings, where obtained from a nursery. Uniform seedlings (300 mm in height) were planted into washed sand in 30L pots in an outdoor nursery site at Lincoln, New Zealand in December 2004. Half of the 80 plants were assigned randomly to a defoliation treatment where 66% of new leaves (i.e., two of each three newly grown pairs) were removed at monthly intervals during the growing season (January to April 2005). The pots were laid out into 20 blocks of four plants, where each block contained two defoliated and control plants. The plants were irrigated as necessary with a nutrient solution following Millard and Proe (1991) using a concentration for nitrogen of 3 mM.

After the start of flowering in February (late summer), mesh bags were placed over all flowers to prevent seed loss (Plate 3.1). On each plant, all the flowers were classified according to their position on main or lateral shoots, their numbers were counted and their lengths measured in late autumn after elongation had ceased (early May).

Plate 3.1 Bagging flowers to prevent seed loss in May 2005. Plants are 6 months old.

Seed samples used in the germination experiment were taken from a representative flower of each shoot type on all plants after the first seed capsules opened in late autumn (May) and air dried. In late winter (August) the number of flowers per shoot was counted for all plants. Two flowers representative of each shoot type were selected from each plant and all the seeds were extracted and dried at 70 ^{o}C to obtain the total seed dry mass per flower and average seed dry mass, *W*, for each shoot type. Seed number per flower was calculated as the quotient of seed mass per flower and *W* for each shoot type. Seed mass for each shoot type was determined from the product of seed mass per flower and flower number. Summation of these values for both shoot types was done to determine total seed mass per plant. Using a similar procedure, with seed number substituted for seed mass, seed number was scaled from the flower to the shoot and plant level.

3.2.2 Leaf area production

Phenological measurements of leaf area production were made over the course of the growing season on a subsample of 40 plants from all 20 blocks (n = 20 per treatment). A representative branch on each plant was selected, each node was numbered and the two leaves occurring at each node were classified as being present or absent through defoliation or natural causes. The measurements were also made on a single representative side shoot selected from the main branch. Leaf area was estimated from measurements of leaf length and width and the number of leaves as described in Chapter 2. The measurements were used to determine shoot level net leaf area (A_n) at the end of the growing season and cumulative emergent leaf area, A_e, over the course of the period of flower and seed formation, from late summer (February) to late winter (August), as described in Chapter 2.

3.2.3 Seed germination

Seed germination was recorded for seeds placed in distilled water in a dark growth cabinet with air temperature set at 25 °C, the optimum temperature and water supply for germination (Jay, 2006). Germination was recorded for seeds from main and side shoots of all 80 plants, giving a total of 160 replicates (80 plants x 2 shoot types per plant). For each replicate, 50 seeds were placed on top of three filter papers in an 85 mm diameter Petri dish. The dishes were sealed with tape in order to minimise evaporation and the seeds were hydrated daily by adding 2 ml distilled water to each dish. The number of seeds germinating (a radicle of 1 mm length had emerged) was counted from the first observation at intervals of 3, 3.5, 4, 4.8, 6 and 8 h, then at every 8 h until maximum germination had occurred.

3.2.4 Data analysis

Cumulative germination, *Gr*, was modelled as a function time, *t*, after the seeds were placed in the dish, using the Weibull function,

$$Gr = Gr_{\max} e^{-(t/b)^c} \quad (1)$$

where $Gr_{\max}$ is maximum number of germinated seeds and *b* and *c* are empirically determined parameters. Differentiation of equation 1 allows the calculation of the maximum rate of seed germination and the time at which this occurs. Rearrangement also allows the calculation of the time taken for the 1st and 99th percentile of seeds to germinate.

All data were analysed using SAS (SAS Institute, 1996). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet these underlying statistical assumptions. The main and interactive effects of defoliation and shoot type on all variables were analysed using a mixed model which accounted for the split plot nature of the experiment. Treatment effects within each shoot type were determined by multiple comparison tests within the mixed model.

3.3 Results

3.3.1 Effects of defoliation on flower and seed production

Compared to control plants, the dry mass of individual seeds, *W*, was reduced by 11 and 4% for main and side shoot flowers, respectively, but these differences were not significant (Table 3.1, Plate 3.2).

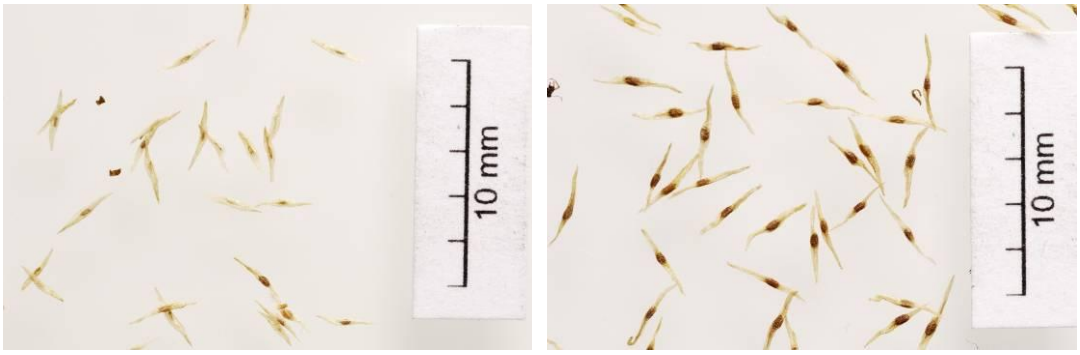


Plate 3.2 The size of Individual seeds of defoliated (left) and undefoliated plants (right).

Defoliation induced a reduction in seed mass per flower of 26% (P = 0.0505), which was primarily attributable to a significant (P = 0.03) decrease in flower length (Table 3.1, Plate 3.3). The number of flowers per shoot was also diminished through defoliation by 9.5% for main and 16.9% for side shoots (significantly at $P < 0.05$ for side shoots). Consequently, total plant seed mass, W_s, in control plants (15.1 ± 2 g) exceeded that of defoliated plants (10.1 ± 1 g) by 35% ($P < 0.05$).

Plate 3.3 Size and seed density of an average main flower of defoliated (left) and undefoliated plants (right).

Compared to the control plants, defoliation reduced the number of seeds produced per single flower ($P < 0.05$) by 14% for flowers on the main shoots and 30% for flowers on the side shoots (Table 3.1, Plate 3.3). Defoliation also induced a substantial reduction in the number of seeds at the shoot (18 and 23% for flowers on the main and side shoots; $P < 0.05$) and the plant level (24 %; $P < 0.05$), where average seed numbers were 4.62×10^5 for control plants and 3.51×10^5 for defoliated plants (Plate 3.4).

Table 3.1 Effects of defoliation on flower- and seed production at the shoot level. Values are means ± standard error in parentheses from 20 blocks and values followed by the same letter are not significantly different at $P < 0.05$.

	Flower length mm	Flower number per plant	Dry mass of single seeds µg	Seed number per flower ($\times 10^{-3}$)	Seed number per plant ($\times 10^{-4}$)
Main shoots					
Treatment	162.4 ± 7.4 a	3.8 ± 0.3 a	29.5 ± 1.3 a	35.5 ± 4.2 a	13.7 ± 2.2 a
Control	178.1 ± 5.4 a	4.2 ± 0.3 a	33.1 ± 1.5 a	41.4 ± 3.8 a	16.6 ± 1.9 a
% loss	8.8	9.5	10.9	14.1	17.5
Side shoots					
Treatment	64.4 ± 4.7 a	20.8 ± 2.0 a	28.2 ± 1.8 a	8.06 ± 1.2 a	21.4 ± 3.6 a
Control	75.9 ± 3.2 b	25.1 ± 2.1 b	29.1 ± 1.4 a	11.4 ± 1.3 b	29.6 ± 4.2 b
% loss	15.2	16.9	3.6	29.5	27.7
Overall treatment effect (P values)					
Defoliation	0.033	0.11	0.18	0.04	0.06
Shoot type	< 0.0001	< 0.0001	0.03	< 0.0001	0.02
Defoliation × Shoot type	0.22	0.20	0.32	0.27	0.14

Plate 3.4 A mature main flower (here control) may contain up to 166 000 wind-dispersed seeds. Buddleia plants produced an average of 29 flowers in total in the first year.

3.3.2 Relationship between leaf area production and reproductive capacity

There was no significant difference in average net leaf area (A_n) per branch between treatments. However, emergent leaf area (A_e) and leaf mass per branch (W_e) over the flowering period (February -August) was significantly ($P < 0.01$) higher in defoliated plants by (89 % and 86 %, respectively) than in the control plants (Table 3.2). On this subsample of plants that were measured for leaf area defoliation reduced total seed production (W_s) significantly ($P < 0.001$) by 42 %. Thus, the ratios of both W_s / W_e and W_s / A_e were significantly lower in defoliated plants by 49 and 50 %, respectively (Table 3.2).

Table 3.2 Effects of defoliation on the ratio of seed to leaf production. Leaf areas shown reflect the flowering period only (February to August 2005). Means ± standard error of a subsample of 20 plants from twenty blocks are shown for net leaf area, A_n, the increase in emergence leaf area, ΔA_e, and mass, ΔW_e, and total seed production W_s. Asterisks ***, ** following *F*-values represent significance at plant level at $P < 0.01$ and $P < 0.05$.

	Control	Defoliated	Significance
A_n (m^2 $branch^{-1}$)	0.11 ± 0.02	0.11 ± 0.02	0.07 ^{ns}
ΔA_e (m^2 $branch^{-1}$)	0.19 ± 0.04	0.36 ± 0.07	5.58 **
ΔW_e (g $branch^{-1}$)	10.2 ± 2.23	19.0 ± 3.71	5.58 **
W_s (g $plant^{-1}$)	16.0 ± 2.84	9.28 ± 2.25	7.68 ***
W_s / A_n	262.2 ± 62.5	224.3 ± 92.7	1.71 ^{ns}
W_s / A_e	168.2 ± 39.8	84.8 ± 39.4	9.69 ***
W_s / W_e	3.2 ± 0.76	1.62 ± 0.76	7.01 **

3.3.3 Effect of defoliation on germination

Neither the main or interactive effects of defoliation treatment and shoot type significantly ($P > 0.05$) influenced any of the parameters describing germination (Fig. 3.1). When averaged across all treatment and shoot types, germination started after 49 h and reached a maximum of 99.7% of the total seed number after approximately 239 h. The average maximum rate of germination was 1 seed h^{-1} and was reached after 68 h.

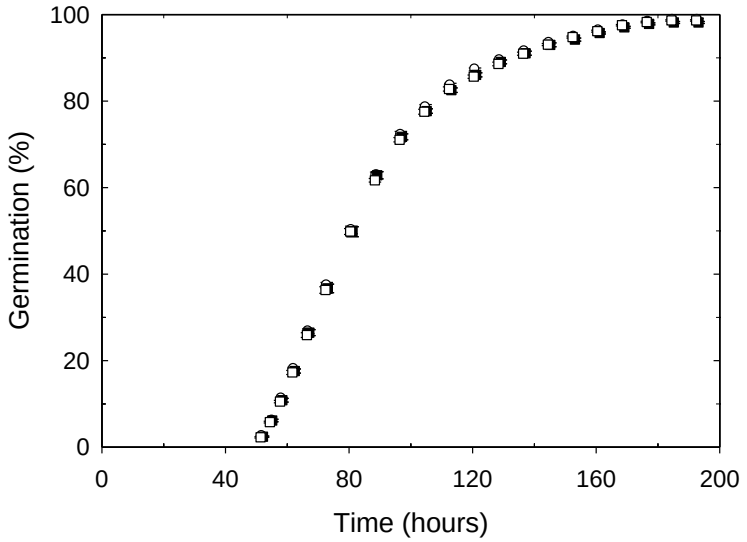


Figure 3.1 Germination time course for seeds from undefoliated main shoots (filled circles), defoliated main shoots (open circles), undefoliated side-shoots (filled squares) and defoliated side shoots (closed squares). Each point shown is the mean ± standard error from twenty blocks. Standard errors are obscured by symbols for all treatments.

3.4 Discussion

3.4.1 Effects of defoliation on reproductive capacity

Defoliation induced a major reduction in the reproductive capacity in *B. davidii.* The results from this study show that despite the high plasticity displayed for vegetative growth, defoliation did not induce a similar compensational response in seed production. This result is consistent with other studies on woody species (Obeso, 1997; , 2002; Kawamura and Takeda, 2006), but somewhat surprising given the invasive vigour of *B. davidii*.

While in woody plants defoliation often results in compensational leaf production concomitant with declining flower and seed production (Tong *et al.*, 2003; Mueller *et al.*, 2005), perennial herbs may compensate in both, leaf area and reproductive output (Obeso, 1993; Mabry and Wayne, 1997; Parra-Tabla *et al.*, 2004). The difference was explained by the fact that herbaceous plants exhibit a higher plasticity in their growth capacity and photosynthesis, and also have lower fruit production costs while woody plants experience greater limitations in assimilate allocation, have longer fruit maturation times and higher costs of fruit production (Obeso, 1993). The cost of reproduction seems to be a major determinant for the response to defoliation. Further evidence can be drawn from studies that compare gender and plant size in dioecious species, where female plants were found to be smaller than male plants in woody species but larger in herbaceous plants (Obeso, 1993; 1997). However, different shoots within the same plant may also experience costs of reproduction. Kawamura and Takeda (2006) report for *Vaccinium hirtum*, that reproductive branches were shorter in comparison to vegetative branches, had fewer side shoots and that the cost of flowering increased with shoot length. It is likely that the distance of sinks (branches or flowers) to sources of assimilates determine resource allocation. Obeso (1993) found that assimilate allocation to sinks closer to the source (assimilating leaves) was higher

and to sinks more distant from the source was lower. If a smaller size of sources would have the same effect as increasing distance, flowers on defoliated branches would likewise experience less provisioning and produce fruit of lower quality or quantity. The reduction in flower and seed production in *B. davidii* in response to defoliation would support this suggestion.

3.4.2 Trade off between reproduction and leaf area growth

Not only the supply of assimilates but also the strength of physiological sinks determines the allocation of assimilates within plants. Repeated defoliation of 66% of newly grown leaf area not only reduced the reproductive capacity in *B. davidii* by about one quarter when compared with the control plants, but also induced considerable compensational leaf area production (Chapter 2), which would constitute an additional sink. In *B. davidii*, side shoots contributed the major proportion to compensational leaf area production after defoliation. Side-shoot flowers may therefore have experienced greater competitive sink strength from compensational leaf growth than flowers on main shoots. Side-shoot flowers were more numerous (5-6 fold) and they contributed about two thirds of the total seed production per plant. This may be the reason why the reductions in seed production were significant in side shoots but not in main shoots.

Weighting leaf production against seed production at plant level shows that the increase in the growth rate of emergence leaf area in defoliated plants was 2-fold greater than in control plants while the production of total seed mass was almost halved. Both effects were significant at $P < 0.05$. Thus, defoliation induced a substantial and significant reduction in the ratio of reproduction to leaf area growth. The results may indicate that different physiological sinks can compete for assimilates which may influence reproductive output. At the shoot level, differences in the defoliation impact on single flowers may

originate from the architectural position of the shoot and may be determined by resource availability. In *B. davidii*, defoliation induced a shift in resources allocation favouring leaf growth over reproduction, which resulted in a considerable decline in seed production.

3.4.3 Effects of defoliation on germination

Although flower and seed production decreased markedly following defoliation, the germination capacity of individual seeds was not affected. Although several studies report similar observations (Aizen and Raffaele, 1997; Apollo *et al.*, 1998), other studies showed that in most woody perennials a reduction in seed mass was associated with a decline in germination rates and seedling survival (Thalmann *et al.*, 2003; Tong *et al.*, 2003; Mueller *et al.*, 2005). However, the species used in these studies (white pine, horse chestnut and mangrove) had rather large seeds in comparison to those of *B. davidii*, which are wind-dispersed and seem not to require a large endosperm for successful germination. (There were signs that some seeds in the defoliated treatment were not filled properly, but unfortunately, this didn't show as a statistically significant difference in the seed weight of individual seeds.)

Nevertheless, both reduced seed numbers and seed quality following defoliation will likely impact on population dynamics of plants and may affect the colonising success of the species over several years (Mueller *et al.*, 2005). In *Atriplex vesicaria*, low seed availability after grazing was responsible for a decline in the plant population (Hunt, 2001) and in horse chestnut, concern was expressed that the long term survival of the species may be endangered following a heavy infestation of leaf mining moths (Thalmann *et al.*, 2003). Despite the still high seed output, the defoliation-induced reduction in flower and seed production in *buddleia* may help to reduce its invasive vigour. It is likely that continued

defoliation with time will further reduced seed numbers and may also reduce seed quality below critical values.

3.5 Summary

B. davidii shows, like most woody species, no compensation in reproduction following defoliation. This study supports the Cost of Reproduction hypothesis and shows how different sinks may compete for assimilates. Defoliation increased resource allocation to leaf production at the expense of reproduction resulting in a considerable decline in both flower and seed production. The reduction of 42% in total seed production per plant will help to limit reestablishment of seedlings and contribute to control the further spread of this invasive weed. The impact of herbivory on seed germination may depend on the magnitude and duration of the damage and the availability of soil nutrients.

4

THE IMPACT OF DEFOLIATION ON NITROGEN TRANSLOCATION PATTERNS IN *BUDDLEIA DAVIDII* FRANCH

4

THE IMPACT OF DEFOLIATION ON NITROGEN TRANSLOCATION PATTERNS IN *BUDDLEIA DAVIDII* FRANCH

4.1 Introduction

Internal cycling of nitrogen (N) is a major source of nitrogen used for the seasonal growth of both evergreen and deciduous trees (Millard, 1996). In temperate ecosystems, N is stored in the perennial tissues of trees during winter and remobilised in spring to supply early leaf growth, either before root uptake of nitrogen occurs (Millard, 1994; Millard *et al.*, 2001; Millard *et al.*, 2006) or concurrently (Malaguti *et al.*, 2001; Millard *et al.*, 2001; Frak *et al.*, 2002). Nitrogen remobilised from storage can supply the majority of N used for leaf growth in spring (Dyckmans and Flessa, 2001; Millard *et al.*, 2001; Carswell *et al.*, 2003) and so is an important physiological process regulating productivity.

The mechanisms of N storage and remobilisation are determined, in part, by leaf habit. Deciduous species store nitrogen during the winter in woody tissues like roots (Tagliavini *et al.*, 1999), stems (Millard *et al.*, 2001) and bark (Cooke and Weih, 2005). In contrast, coniferous (Nambiar and Fife, 1987; Millard and Proe, 1993) and broad-leafed evergreen species (Wendler *et al.*, 1995; Cherbuy *et al.*, 2001; Millard *et al.*, 2001) store nitrogen in leaves rather than woody tissues. The pattern of nitrogen remobilisation in semi-

deciduous species is less clear. For example, *Nothofagus fusca* combines deciduous (N stored in roots) with evergreen characteristics (N remobilisation independent of leaf senescence and continued nitrogen uptake from soil during the winter) to maximise nitrogen acquisition and productivity (Stephens *et al.*, 2001). *Citrus sinensis* stores nitrogen mainly in leaves and, to a lesser extent, in woody tissues (Legaz *et al.*, 1995). The physiological mechanisms regulating these patterns are not thoroughly understood.

The invasive shrub *B. davidii* has no special requirements for N supply and thrives vigorously in poor soils (Miller, 1984; Humphries and Guarino, 1987). In New Zealand, it is successful as a primary coloniser in riverbeds (Bellingham *et al.*, 2005). While this indicates a high N-use efficiency, it also suggests that storage of N might be particularly important for spring leaf growth in *B. davidii*.

In 2006 a leaf chewing weevil has been released in the context of biocontrol of this invasive species (Brockerhoff *et al.*, 1999; ERMA, 2005). Like many other species with an indeterminate growth pattern (Molvar *et al.*, 1993; Danell *et al.*, 1997; Hester *et al.*, 2004), *B. davidii* is capable of compensatory leaf growth in response to defoliation (Chapter 2). This is mediated partly through increased node production and leaf size, but also though increased allocation of resources to new leaves (Watt *et al.*, 2007). Such defoliation-induced compensatory growth constitutes an increased sink strength (Kim *et al.*, 1991). Additional N could be supplied either through increased root uptake (Jonasson, 1995; Raillard and Svoboda, 1999; Millard *et al.*, 2001) or from storage. Thus, the balance between remobilisation and root uptake of N is important in relation to any compensatory growth response. The aim of this study was to determine (i) the impact of defoliation on the growth of *B. davidii;* (ii) the site of N storage and (iii) the extent to which N remobilisation was affected by defoliation. It is hypothesised that repeated defoliation of new leaf area would

increase remobilisation of N from storage, resulting in increased root uptake of N to replenish depleted stores.

4.2 Methodology

4.2.1 Experimental design and treatment description

In late spring (December) 2004, eighty *B. davidii* seedlings 300 mm in height were planted into 30 L pots containing washed sand. The plants were grown for the period of a year (two growing seasons) in a sheltered nursery at Lincoln, New Zealand, with an average daily mean temperature of 12.4 ^{o}C. Forty of the plants were manually defoliated by removing 66% of newly grown leaf area. The defoliation treatment was applied four times in each growing season at monthly intervals from January to April during the first season (17 January - 14 June 2005) and from October to December during the second season (28 June - 28 December 2005) (Table 4.1). The pots were laid out in a randomised block design with a total of 40 blocks. Each block contained one defoliated and one undefoliated plant.

The plants were irrigated twice per week with a nutrient solution following Millard and Proe (1991) using a medium concentration for N of 3 mM and additional water was supplied to ensure adequate irrigation. During the first season the plants were supplied with N at natural ^{15}N abundance. At the start of the second season (August 2005), when spring growth was about to commence, all pots were flushed 3 times with water and the nutrient solution was changed to include labelled nitrogen with ^{15}N at 10 atom% enrichment. At this time the last leaves grown in the previous season were marked with white paint to distinguish “old tissue” (grown in the first season with ^{14}N supply only) from “new tissue” (grown in the second season with ^{15}N supply).

Table 4.1. Description of time since labelling, date of defoliation and harvest during the experiment.

	Time of year	Date of defoliation	Date of harvest	Labelling days
First season	Summer	17-Jan-05	-	-
	Summer	09-Feb-05	-	-
	Summer	09-Mar-05	-	-
	Autumn	06-Apr-05	-	-
Second season	Winter	-	26-Aug-05	0
	Spring	-	27-Sep-05	32
	Spring	04-Oct-05	18-Oct-05	53
	Spring	26-Oct-05	7-Nov-05	73
	Summer	01-Dec-05	4-Dec-05	100
	Summer	28-Dec-05	6-Jan-06	133
	Summer	-	28-Jan-06	155
	Summer	-	27-Feb-06	185

4.2.2 Plant harvest and calculations of nitrogen content

Initial values of biomass were obtained from harvesting six seedlings in December 2004 at the start of the experiment. During the second season, eight monthly harvests of five plants per treatment were undertaken, beginning with the first harvest prior to changing the source of nitrogen, to obtain initial values before labelling. For each harvest, plants were chosen which best represented the average leaf area of each treatment at that time. All components were separated, divided into "old tissue" and "new tissue" and dried at 70 ^{o}C until constant mass was reached, then weighed and ground (Plate 4.1). For defoliated plants the mass of defoliated leaves was added to the harvested leaves to obtain total dry mass of leaves. Roots

were washed gently in water before drying. Total N content and ^{15}N abundance in samples of all tissues were determined by isotope ratio mass spectroscopy at the Macaulay Institute, Aberdeen UK. The amount of labelled N taken up by the roots and the amount of remobilised N per plant were calculated as described in Millard and Neilsen (1989).

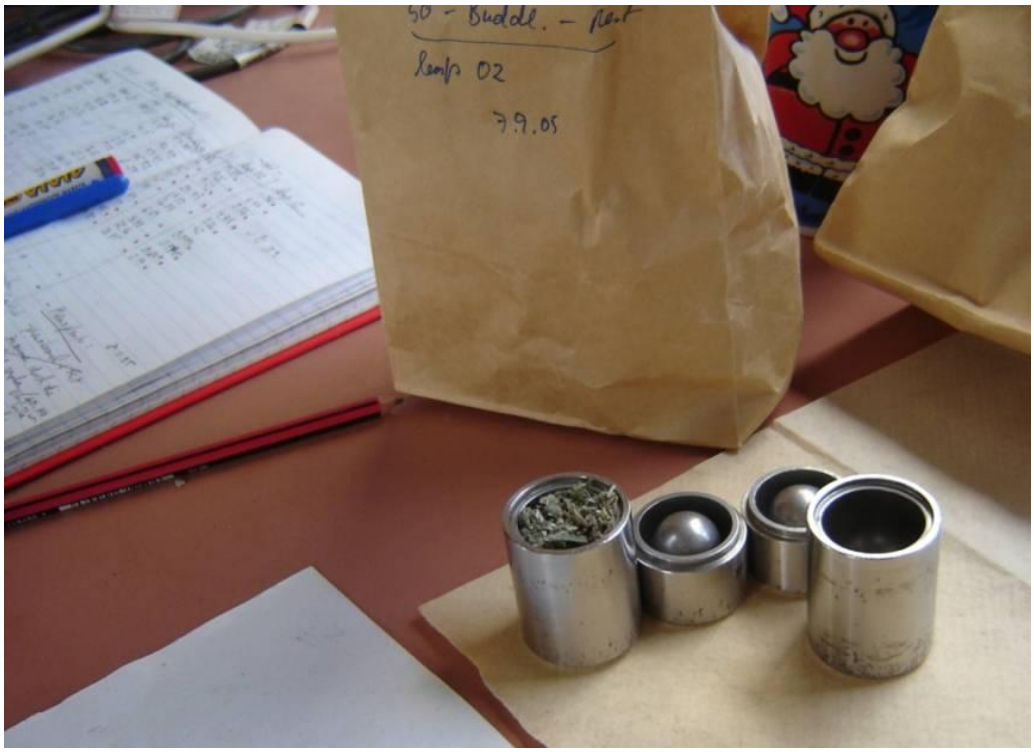


Plate 4.1 Preparing tissue samples for stable isotope analysis.

4.2.3 Statistical analysis

All data analysis was undertaken using SAS (SAS Institute, 1996). The main and interactive influence of defoliation and time of the year on dry mass, nitrogen content and concentration was analysed using general linear models. Variables were tested for normality and homogeneity of variance and log- and square root-transformations were made when necessary to meet the underlying assumptions of the general linear model used.

4.3 Results

4.3.1 Plant growth

Defoliation resulted in substantial reductions (26 %) in the mean total dry mass of whole plants at the final harvest. The overall treatment effect of defoliation on dry mass was significant at the whole plant level ($P < 0.0001$; Fig. 4.1a) and for all tissues ($P < 0.03$; Fig. 4.2). Total leaf dry mass in defoliated plants at the start of summer (day 100), when the control plants reached maximum values, was 37% lower for new leaves ($P < 0.03$) and 63% lower for old leaves ($P < 0.01$). At the final harvest there was a reduced biomass of stems, flowers (both 31 %), total above ground woody tissue (21 %) and roots (39 %) of defoliated plants ($P < 0.001$). A short period of hot and dry weather resulted in a single transient decline in dry mass in both treatments around day 155.

4.3.2 Defoliation impact on nitrogen content and translocation in whole plants

For whole plants, the total amount of unlabelled N was on average 32% lower in defoliated plants ($P < 0.0001$) and clearly reflected the smaller plant size (Fig. 4.1b). A significant interaction of defoliation and time was observed for labelled N ($P < 0.0001$), indicating that defoliation had affected the pattern of N uptake. In defoliated plants up to 57% more (day 53; $P < 0.0001$) labelled N was taken up by the roots during spring, when compared with the control, despite their lower dry mass. As a result, there was no significant difference in the total N content ($P > 0.08$) between treatments during spring despite the considerable reduction in dry mass of defoliated plants (Fig. 4.1a).

The differences in plant size may obscure a defoliation effect on N translocation and uptake. Therefore, the concentration of N per unit dry mass was chosen to correct for different plant size (Fig. 4.1c). The overall treatment effect and the interaction of defoliation

and time was significant ($P < 0.0001$) for the concentration of labelled and total N of whole plants. The concentration of labelled N per unit dry mass was significantly greater in defoliated plants during most of the season ($P < 0.0001$), but especially so in spring (140% on day 53), indicating increased root uptake. Accordingly, values of the total N concentration in whole plants were also significantly higher (34 %) during that time. The contribution of labelled N to the plants total N increased in defoliated plants in comparison to control plants ($P < 0.0001$ for overall interactive and treatment effects) and averaged 23 vs. 16% in spring and 59 vs. 53% in summer, for defoliated and control plants, respectively. Although the total concentration of unlabelled N was not substantially different between treatments, a significant interaction of defoliation and time ($P < 0.002$) was obtained for unlabelled N as well.

Figure 4.1 The influence of defoliation on a) total biomass, b) nitrogen content and c) nitrogen concentration per unit dry mass for whole plants of defoliated (●) and undefoliated (○) plants. Shown are means ± standard error (n = 5) of total nitrogen (full lines), unlabelled nitrogen (dotted lines) and labelled nitrogen (dashed lines) in b) and c). Note the different scales on the Y-axis.

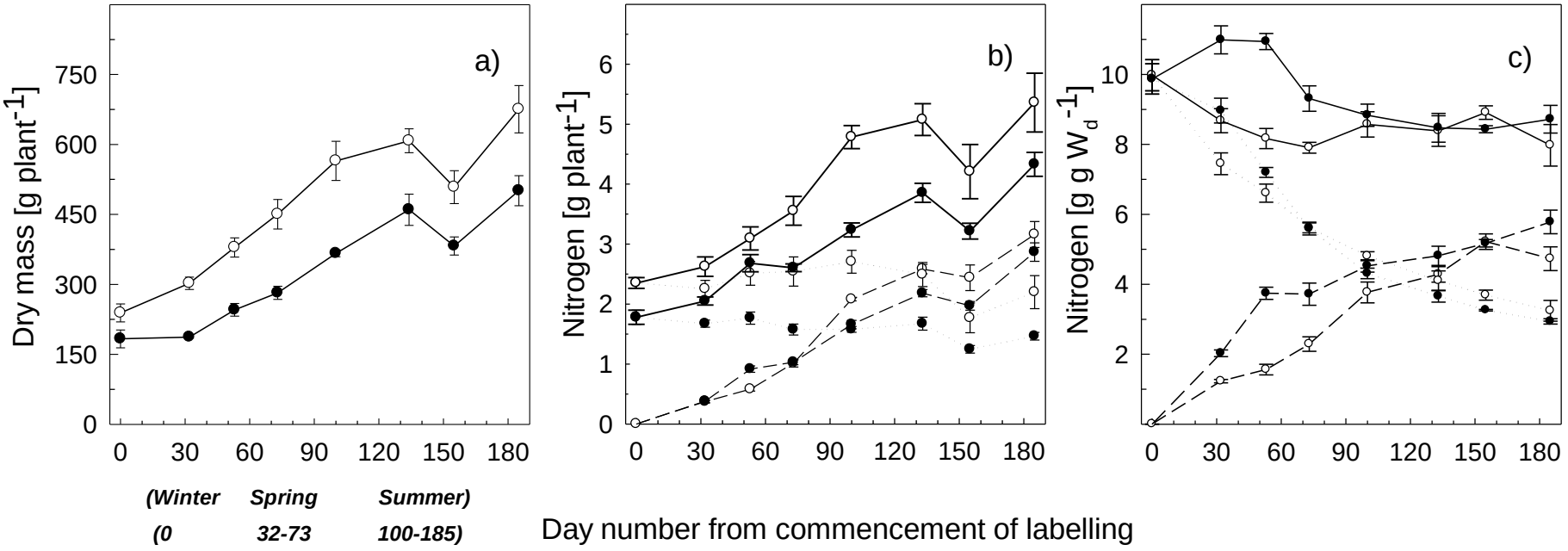
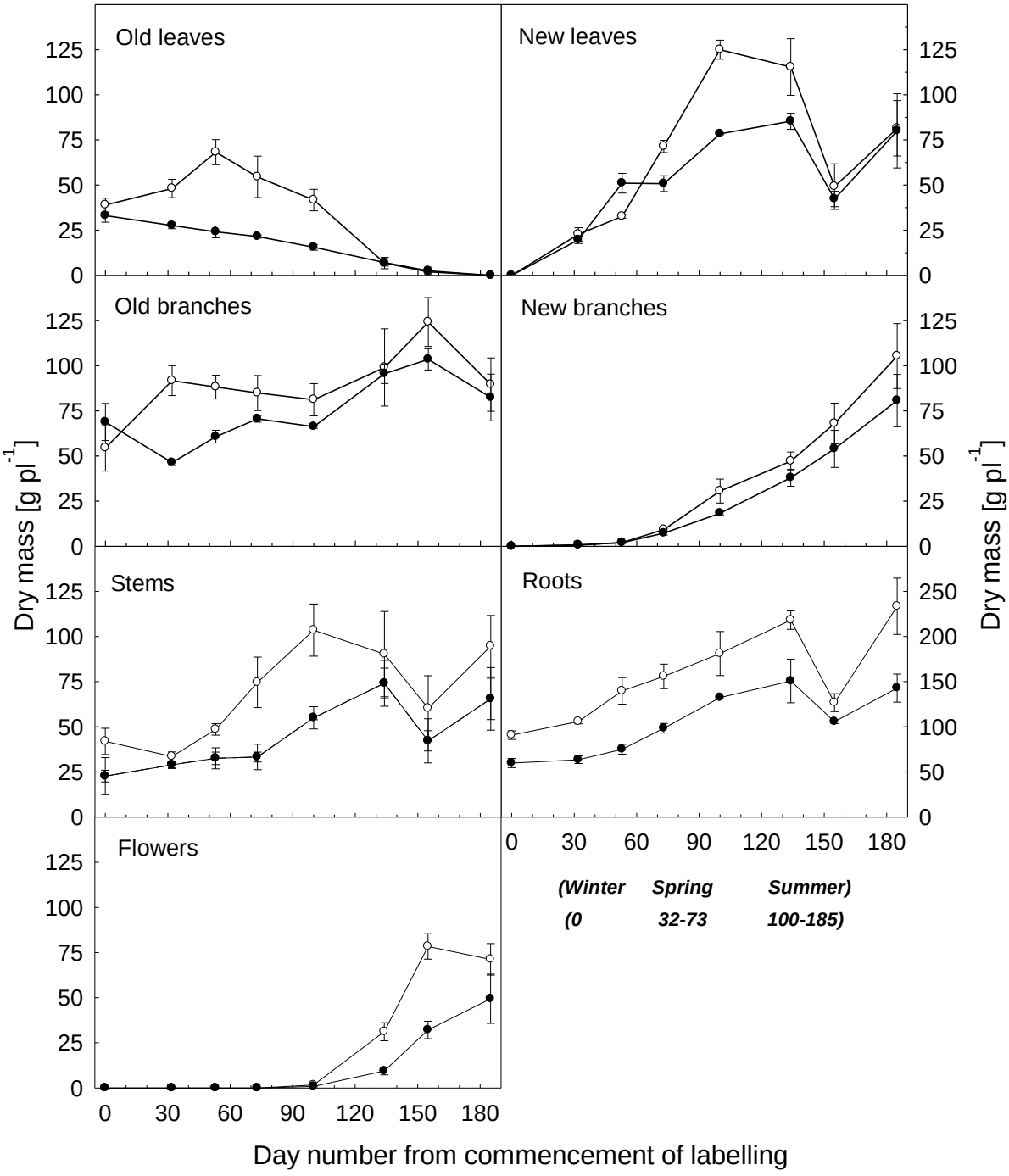


Figure 4.2. Seasonal changes in dry mass of plant tissues for defoliated (●) and undefoliated (○) plants. Values represent mean ± standard error of five plants. Note the different scales on the Y-axis.



4.3.3 Site of nitrogen storage and remobilisation

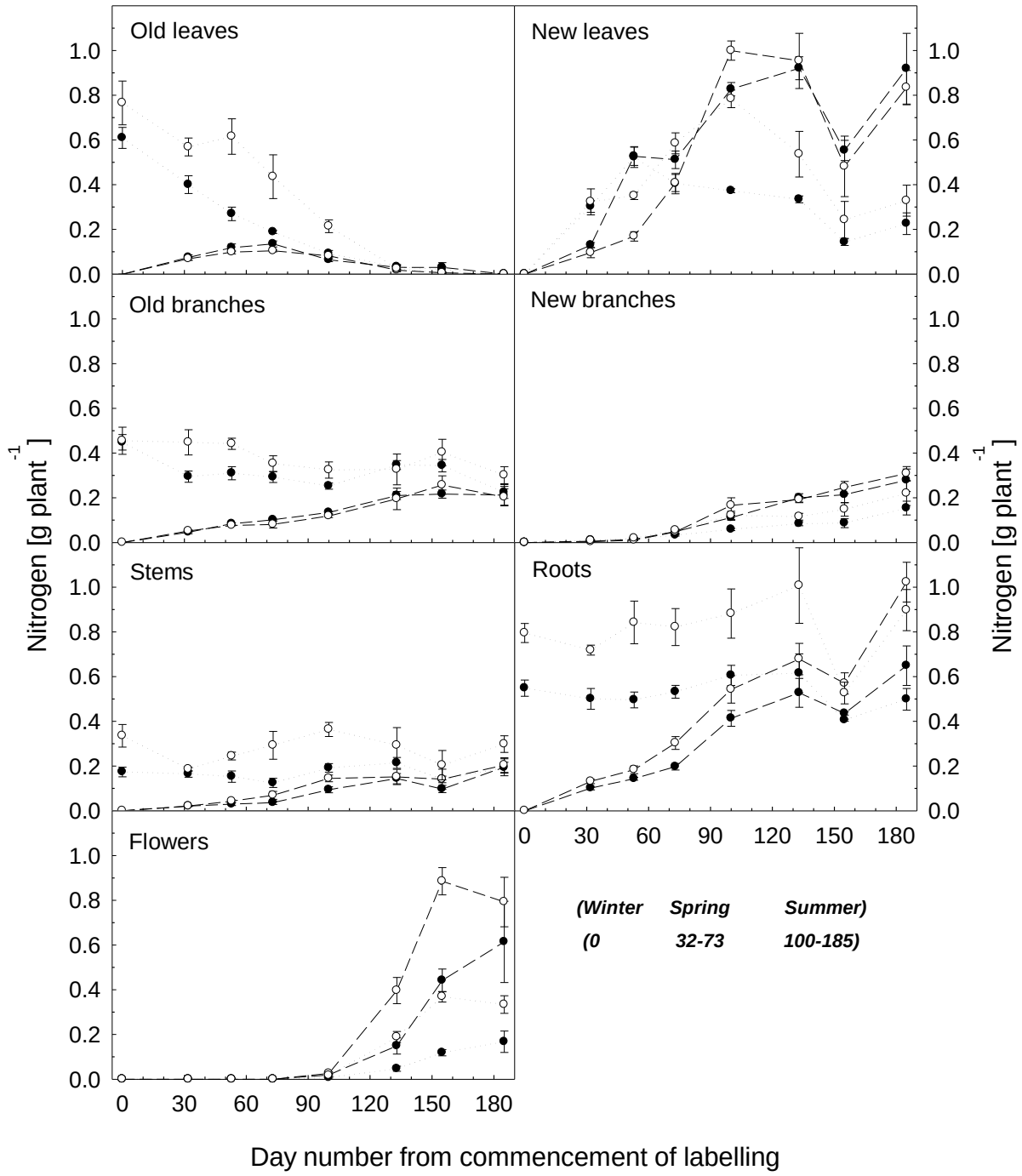
In both treatments, unlabelled N declined in old leaves ($P < 0.0001$; Fig. 4.3). From maximum values in winter (34% of total N for both treatments) unlabelled N steadily declined to zero in late summer (day 155). In the undefoliated plants, none of the woody tissues remobilised any N ($P > 0.1$) so that about the same amount of unlabelled N was recovered in new leaves. In defoliated plants, additional N was remobilised from old branches ($P < 0.0002$) accounting for the small discrepancy in the amount of remobilised N between old and new leaves. As a proportion of the total plant nitrogen, remobilised N in old branches declined from higher values (25 vs. 19% of total N in defoliated and control plants, respectively) at the start to values similar to the control at the end of harvest (5 %).

4.3.4 Nitrogen allocation at the tissue level

Both labelled and unlabelled N that was taken up by the roots was mainly supplied to new growth of leaves and flowers. Only these two tissues showed any increase in unlabelled N and accumulated the highest amount of N taken up by the roots as a proportion of the total nitrogen. Details of the defoliation-induced changes in uptake and remobilisation patterns suggested in Fig. 4.1c are explained in Fig. 4.3.

In new leaves of defoliated plants, the maximum amount of unlabelled N was attained 47 days earlier (absolute values were 50% greater in comparison to the control; day 53, $P < 0.017$) and also represented a higher proportion of the total N (20 vs. 16 %) compared to control plants at that time. The stronger remobilisation of nitrogen in spring was followed by considerable decline during summer in the defoliated treatment (absolute values in defoliated plants being on average 41% lower than those of control plants).

Figure 4.3 Seasonal changes in labelled (dashed lines) and unlabelled (dotted lines) nitrogen content of plant tissues for defoliated (●) and undefoliated (○) plants. Values represent mean ± standard error (n = 5).



Uptake of unlabelled N in spring increased with total values exceeding those of control plants by two-fold (day 53, $P < 0.0001$). During summer the N uptake slowed in defoliated plants, while it continued exponentially in the control. Maximum values of uptake were attained in mid summer and represented a greater proportion of the total of N in defoliated plants when compared to control plants (26 vs. 21 %). Over the remainder of summer quantities of labelled N were similar between treatments. The amount of labelled nitrogen in woody tissues was not different over the whole season, despite the considerable reduction in dry weight of defoliated plants. In defoliated plants, a consistently smaller proportion of both labelled (14 vs. 20% of total nitrogen) and unlabelled N (4 vs. 8% of total nitrogen) was allocated to flowers in comparison to control plants.

The concentration of N per unit dry weight (labelled and total N) was also significantly higher ($P < 0.0001$) in both old and new leaves of defoliated plants (Fig. 4.4). In spring (day 53), values exceeded those of the control by 107 and 32% for labelled and total N, respectively, in new leaves and by 231 and 57% in old leaves. The concentration of unlabelled N was significantly higher ($P < 0.0001$) in old leaves during spring (indicating N remobilisation from storage), while in new leaves higher concentrations were reached in spring followed by lower concentrations over summer ($P < 0.01$). In flowers (not shown) values were 18% higher for labelled N, but 27% lower on average for unlabelled N with ($P < 0.0001$).

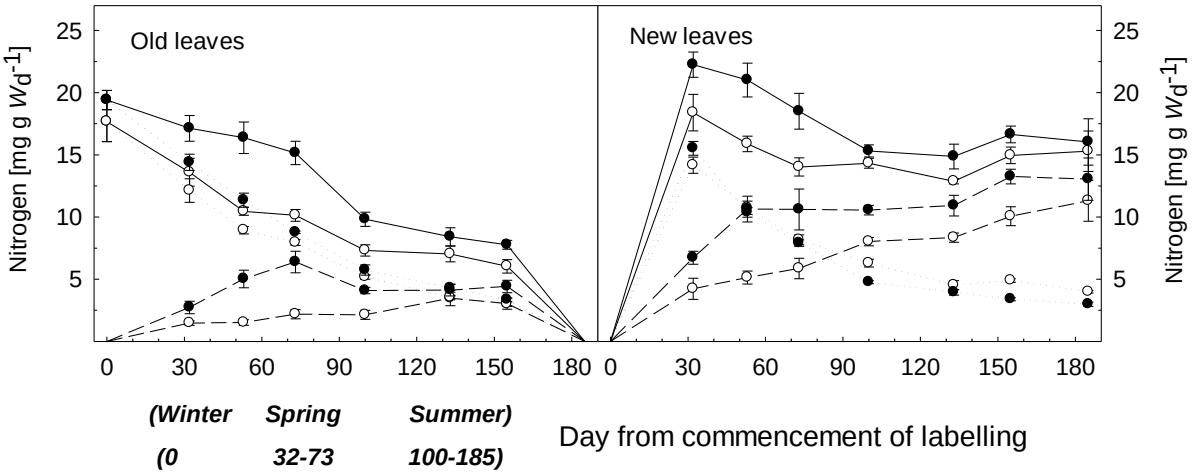


Figure 4.4 Seasonal changes in labelled (dashed lines), unlabelled (dotted lines) and total (full lines) nitrogen concentration in old and new leaves of defoliated (●) and undefoliated (○) plants. Values represent mean ± standard error (n = 5).

4.4 Discussion

4.4.1 Plant size and compensational growth

Defoliation caused a 26% reduction in the total plant dry mass, which reflects the negative influence of continued leaf removal and the resulting reduced assimilatory capacity on plant size. The decline in biomass production, especially that of roots (39 %) and flowers (31 %), has important implications for the efficacy of defoliation as a control measure, as smaller roots suggest a reduced capacity for uptake of nutrients from the soil and reduced flower production may assist in reducing the invasive spread of the species.

4.4.2 Amount and site of nitrogen remobilisation

The source of remobilisation of N in *B. davidii* differs from that of other semideciduous species (*Citrus sinensis* remobilises N from old leaves and woody tissue (Legaz *et al.*, 1995) while *Nothofagus fusca* nitrogen is remobilised mainly from roots (Stephens *et al.*, 2001)) in that N is remobilised from old leaves only; no contribution of nitrogen came from woody tissue in undefoliated plants. This is an evergreen characteristic and seems to indicate that N translocation in semideciduous plants is not restricted to a definite type but may occupy a position in the range of characteristics between deciduous and evergreen plants. However, defoliation induced additional mobilisation of N from old branches. This indicates that the reduced pool size of stored N was not sufficient to meet the demands of the increased sink of compensatory leaf growth. While this finding highlights that the physiological functioning in plants in response to environmental stress is highly plastic, it has important implications regarding biocontrol management of this invasive species. New growth early in the season depends largely on stored N, but the amount of N that can be released is determined by the pool size of the tissue stores (Millard and Proe, 1993; Grelet *et al.*, 2003). Thus, in species that mainly store N in leaves, ongoing defoliation will greatly diminish their storage capacity. This may also apply to specimens growing under more severe climatic condition. Although a large part of the foliar N is recovered before leaf abscission in winter, the total leaf mass reduced by herbivores will still be smaller, so that the amount that can be retrieved may also be smaller. Defoliation of semideciduous plants may therefore weaken the foundation for future spring growth, while at the same time compromising the plant's assimilatory capacity.

4.4.3 Defoliation influence on nitrogen uptake and remobilisation

In *B. davidii*, defoliation has previously been found to induce compensatory leaf growth (Chapter 2). From an initial increment of 52% in emergence leaf area production in the first season the surplus (compared to control plants) still reached 15% during the season of the labelling experiment. This study shows that the proportion of remobilised N which supplied new leaf growth during early spring was greater in defoliated plants in comparison to the undefoliated control. This supports observations that the extent of the remobilisation of N is also governed by the sink strength (Kim *et al.*, 1991; Salifu and Timmer, 2003). Since the available pool of stored N in defoliated plants would already have been diminished by the previous season's defoliation treatments, the declining relative contribution of remobilised N to new leaf growth during summer (in comparison to undefoliated plants) confirmed the expectations.

The extent to which soil uptake is affected by defoliation also depends on the site of N storage. Millard *et al.* (2001) showed that defoliation of the evergreen *Pinus sylvestris* (which stores N in young leaves) reduced remobilisation significantly and also affected soil uptake, while in deciduous trees, where the pool of stored nitrogen is not diminished, re-mobilisation and uptake were unchanged. Uptake in *P. sylvestris* was reduced, which was explained by the fixed growth pattern in coniferous evergreens (which does not allows for compensatory growth) and thus lower uptake results from low sink strength. In contrast, many deciduous plants are capable of compensation after defoliation (Millard *et al.*, 2001), which was now also observed for the semideciduous *B. davidii*. Since both soil uptake and storage contribute to the N used for plant growth during most of the growing season, a dwindling supply from storage should induce greater soil uptake to meet the demands, especially in plants where growth is undiminished. Such increment in soil uptake has been confirmed for *Rhododendron lapponicum* and *Carex aquatilis* (Jonasson, 1995; Raillard and

Svoboda, 1999). In *B. davidii* defoliation significantly increased the importance of soil uptake to support new leaf growth, clearly evident in the increased proportion of N uptake by the roots to total plant N over the entire season. In New Zealand, the species generally invades environments that are poor in soil N. Where reduced root development and low soil N supply does not allow a build up of sufficient new N reserves, continued defoliation will diminish the current pool of tissue nitrogen. Thus, it can be predicted that the impact of defoliation in limiting the growth and spread of *B. davidii* should increase with time.

4.5 Summary

B. davidii remobilises N from old leaves only, like evergreen species and thus differs from other semideciduous species. This suggests that, in contrast to deciduous and evergreen species, for semideciduous species the tissue for nitrogen storage and remobilisation is not strictly specified. That N is stored in leaves has important implications for the future growth of defoliated plants. N for new growth is largely supplied from storage, especially where soil supply is insufficient, but defoliation had greatly diminished the pool size of tissue resources. High compensational leaf growth increased the demand for N, which could not adequately be supplied through remobilisation. Thus, defoliation induced additional remobilisation from wood, temporal shifts in nitrogen remobilisation pattern and increased the importance for soil uptake. While this highlights the fact that physiological processes such as nitrogen translocation are highly plastic in response to environmental stimuli, it also has implications for the success of biocontrol of this invasive species in the natural environment. While herbivory significantly reduces the storage capacity for nitrogen, it also considerably affected root growth, which will reduce the capacity for soil uptake. Where the

species invades infertile soils that do not allow quick replenishment of N stores from root uptake, the tendency towards compensational growth in this species should accelerate the success of biocontrol.

5

DO INTERACTIONS BETWEEN *B. DAVIDII* AND A DOMINANT NATIVE NITROGEN FIXER INFLUENCE SOIL NUTRIENT AVAILABILITY AND PLANT GROWTH IN PRIMARY SUCCESSIONS?

5

DO INTERACTIONS BETWEEN *B. DAVIDII* AND A DOMINANT NATIVE NITROGEN FIXER INFLUENCE SOIL NUTRIENT AVAILABILITY AND PLANT GROWTH IN PRIMARY SUCCESSIONS?

5.1 Introduction

A major driver of global change is the increasing number of non-native species that have invaded into natural environments (Vitousek *et al.*, 1997). In New Zealand, more than half of the current flora is comprised of naturalised non-native plant species (Wilton and Breitwieser, 2000). Many of these non-native species have become invasive, for example, the New Zealand Department of Conservation currently manages ca. 340 non-native weed species, and about 7 new species are added to this list annually (DOC, 2007). These invaders are widely thought to pose a threat to indigenous vegetation and habitats by suppressing native species or introducing novel biological functions into indigenous habitats (Smale, 1990; Bellingham, 1998; Standish *et al.*, 2001; Tallent-Halsell and Walker, 2002; Bellingham *et al.*, 2005). Invasive weed species can be strong competitors for light (Richardson *et al.*, 1996; Standish *et al.*, 2001), space (Bellingham, 1998), water (Watt *et al.*, 2003a) or nutrients (D'Antonio *et al.*, 1998; Cathcart and Swanton, 2004). Invaders can also affect ecosystem properties such as soil nutrient availability through differences in litter

quantity or quality (Allison and Vitousek, 2004; Blank and Young, 2004; Rothstein *et al.*, 2004) which may subsequently lead to changes in species composition (Evans *et al.*, 2001), species-specific competitive or facilitative interactions (Peltzer and Wilson, 2001), and alteration of successional pathways by replacement of native species (Smale, 1990).

While short-term alterations to ecosystem processes by non-native species are well documented, data supporting the persistent or long-term impacts of invasive species at the community or ecosystem level is lacking (Levine *et al.*, 2003). For example, acceleration of natural succession including invasive *Buddleia davidii* may be an ephemeral effect because the invader itself is replaced by native forest in later successional stages owing to its relatively short lifespan (ca. 30 yr) (Smale, 1990). Also, despite observing strong increases in soil total phosphate (P) with increasing abundance of buddleia, Bellingham *et al.* (2005) found no influence of *B. davidii* invasion on native or exotic species richness in plant communities. To understand the possible long term impacts of weed invasions it is therefore important to link invader effects with the mechanism by which the effect is conveyed and whether this has consequences for the community (Levine *et al.*, 2003).

An important factor shaping community structure is species interactions. Plant growth is the product of both competitive and facilitative effects between neighbours and differences in growth among species influence dominance-diversity relationships and population demographic processes. The balance of competitive and facilitative interactions among plants may change with life stage and physiology of the partners, but also with plant density, the intensity and importance of abiotic stress (Callaway and Walker, 1997) and indirect effects from additional species (Miller, 1994). This complex relationship may not be sufficiently reflected in glasshouse experiments, emphasising the need for field studies. Walker and Chapin (1987) found that facilitative effects of the nitrogen fixing shrub *Alnus tenuifolia* on the growth of several tree species in a glasshouse experiment were overridden

by negative effects of root competition and shading in the field. Shifts between facilitation and competition can occur along environmental gradients, such as temperature (Callaway and King, 1996), moisture and light (Holmgren *et al.*, 1997) or even between different ecosystems for the same species (see Chapin *et al.* (1994) for positive net effects of *Alnus* in a different environment in contrast to Walker and Chapin (1987)). Often, positive and negative effects occur at the same time between interacting species. For example, Walker and Vitousek (1991) found facilitative (enriched soil nitrogen, seedling shading) and suppressive direct effects (root competition) of the invasive nitrogen fixing tree *Myrica faya* on the native tree *Metrosideros polymorpha* in Hawaii. While differing direct effects play a role in shaping the community structure, it is the net effect that is of main interest for growth and survival of neighbouring species.

Coexisting plants may facilitate each other through increased availability of nutrients. Thus, facilitative effects on nutrient status and growth of plants from nitrogen fixers (also reflected by the great number of nitrogen fixing species used in agriculture (Rao *et al.*, 1998; Baldani and Baldani, 2005)) on neighbouring plants can be strong (Bellingham *et al.*, 2001; Walker *et al.*, 2003; Forrester *et al.*, 2006). *Coriaria arborea* Lindsey, a dominant native nitrogen fixer in primary successions in New Zealand, facilitated growth of the coexisting native shrub *Griselinia littoralis* up to six-fold through increasing soil N (ten-fold) and P (three-fold) (Walker *et al.*, 2003).

It has been hypothesised that in harsh environments the importance of facilitation increases (Bertness and Callaway, 1994). This was supported by Chapin *et al.* (1994), who observed facilitation of neighbours from nitrogen fixing alnus under low nutrient availability, while Walker and Chapin (1987) report increased competition from alnus at higher soil fertility. Thus, nitrogen fixing plants play an important role as ecosystem engineers by greatly increasing soil N (Crews *et al.*, 2001; Vitousek *et al.*, 2002a),

particularly early in nutrient poor soils like early successions on floodplains (Rhoades *et al.*, 2001; Walker *et al.*, 2003). True primary successions are best suited as a system to study facilitation and soil impacts of invasive species. The young substrate has no legacy effects, so that observation of strong effects can be expected.

In contrast to the well-documented impacts of nitrogen fixing plants (both native and non-native), *B. davidii* has been shown to have major effects on both soil N and P by deposition of relatively nutrient rich litter (Matson, 1990; Bellingham *et al.*, 2005). Of particular interest here is the high foliar P because phosphate availability often limits nitrogen fixation (Vitousek, 1999; Uliassi and Ruess, 2002). There is ample evidence that high amounts of P, especially relative to nitrogen supply, increases nitrogen fixation in many species (Hingston *et al.*, 1982; Eisele *et al.*, 1989; Smith, 1992; Vitousek *et al.*, 2002a; Weiss *et al.*, 2005; Benner *et al.*, 2007). A very common scenario is the coexistence of the invasive buddleia with a native nitrogen fixer, *C. arborea* in primary successions in New Zealand, where these two species can be overwhelmingly dominant, together comprising > 80% of the total aboveground plant biomass (Bellingham *et al.*, 2005). I hypothesised that an overyielding effect may increase the invasive spread of buddleia: deposition of high-phosphate litter from buddleia may increase nitrogen fixation in coriaria which in turn may lead to increased facilitation of the invader through increased soil N (Plate 5.1). This would be of concern, especially in primary successions which are very low in both N and P supply (Uliassi and Ruess, 2002). This study investigates the possible facilitative effects between the co-occurring native nitrogen fixer *C. arborea* and *B. davidii* and their impact on soil nutrient availability by comparing growth, foliar nutrients and the availability of soil nutrients in monocultures and mixed stands of both species in field conditions.

Plate 5.1 Facilitation? In the natural environment, specimens of *B. davidii* (brown stems) and of *C. arborea* (green compound leaves) often grow in very close proximity as buddleia seeds accumulate under the canopy of coriaria and do not get washed away by floodings.

5.2 Methodology

5.2.1 Study site

The study site was located on flood plain terraces of the Hapuku (42^{o} 18’ S, 173^{o} 41’ E, 128 m a.s.l.) and Puhi-Puhi rivers (42^{o} 16’ S, 173^{o} 43’ E, 142 m a.s.l.), on the eastern South Island of New Zealand (Figure 5.1). The rivers are surrounded by the tectonically highly active Seaward Kaikoura mountain range (up to 2600 m a.s.l.)(Van Dissen and Yeats, 1991). Uplift rates of the near Hope Fault, which stretches from Hokitika to the coast north of Kaikoura, are about 4 to 6 m ky^{-1}. The underlying parent material is highly fractured

greywacke sandstone with some argillite and tuffaceous sandstone. High erosion of the surrounding slopes leads to the accumulation of exceptionally large amounts of sediment (up to 5 000 t km^{-2} yr^{-1}) in the draining river beds (O'Loughlin and Pearce, 1982; Mackay, 1984). The quality of the sediment in the river beds ranges from stones and coarse sand with no organic layer (open and young terraces) to finer sand (under vegetation on older terraces) and is generally low-moderate in nutrients (Smale, 1990; Vitousek, 1999; Bellingham *et al.*, 2005). The mean soil bulk density for the river beds was estimated with 0.47 ± 0.03 g cm^{-3} which is low and reflects high quantities of coarse, loose material. Mean annual precipitation is about 2 m (Leader, 2005). Mean annual temperature on the Kaikoura coast is 12.1°C. The mountain slopes surrounding the study system are densely covered with native secondary forest (*Kunzea ericoides, Melicytus ramiflorus, Sophora microphylla*, *Olearia paniculata*, *Griselinia littoralis* (Wardle, 1971)) but the river beds are sparsely vegetated because of frequent flooding. *Buddleia davidii* and *Coriaria arborea* are the overwhelmingly dominant pioneer species following severe flood disturbance (Walker and Del Moral, 2003; Bellingham *et al.*, 2005). This study system represents a frequently disturbed primary succession that is common in New Zealand and representative of systems elsewhere following catastrophic disturbances (Walker and Del Moral, 2003).

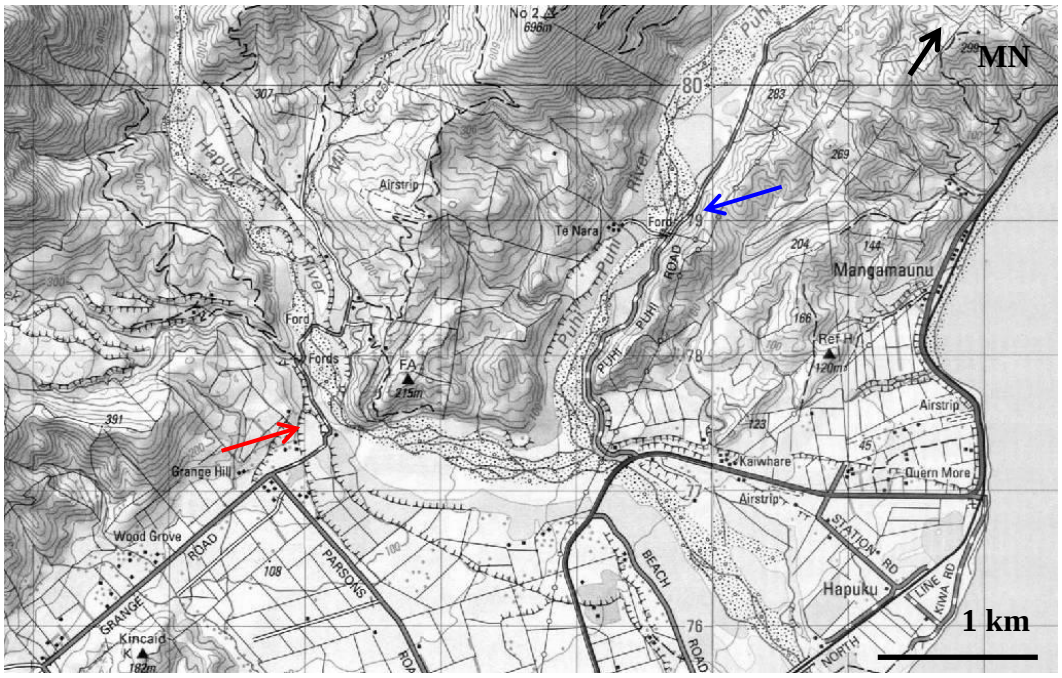


Figure 5.1 Study sites in the Hapuku- (red) and Puhi-Puhi river bed (blue) near Kaikoura Mountain Range. (Topomap Metamedia LtD; Scale: 1: 50000 on the NZGI)

5.2.2 Study species and experimental design

Buddleia davidii Franchet (Buddleiaceae) is a perennial, semi-deciduous, wind-dispersed shrub, native to China, which is well adapted to thrive on disturbed, phosphate- and nitrogen-poor soils (Humphries *et al.*, 1982; Miller, 1984). *Coriaria arborea* (Coriariaceae) is a perennial, evergreen, water- and bird-dispersed nitrogen fixing shrub of up to 6 m in height and is endemic to New Zealand (Bellingham *et al.*, 2005) although the genus is widespread. Both species are common in primary succession on flood plains in New Zealand (Smale, 1990; Gibb, 1994; Clarkson *et al.*, 2002; Bellingham *et al.*, 2005).

In August 2005 (austral midwinter) plots (1m diameter) containing one of four treatments were randomly selected: (a) buddleia, (b) coriaria (Plate 5.2), (c) both species (Plate 5.3) and (d) neither species (control). Random selection was possible as a recent heavy flood (January 2002) left no legacy effect regarding soil nutrients so that all terraces can be considered true primary successions (Bellingham *et al.*, 2005). Also, both species show no

preference for any microsites during their establishment on these surfaces (Walker *et al.*, 2006), so establishment is largely at random. Six replicates for each treatment were set up in each of two rivers; the Hapuku (two terraces) and the Puhi-Puhi (three terraces), giving a total of 48 plots with 12 replicates per treatment. The terraces on each river were no more than 20 m apart to guarantee similar soil substrate. The locations of the plots were scattered throughout the terraces to represent the full range of vegetation density and plant size. The influence of the surrounding vegetation (buddleia and coriaria plants outside the sample plots) was assessed with labelling plots as "low", "medium" and "high" density according the approximate density of the adjacent vegetation and were later included as categorical variables in the statistical analysis. Single species plots were positioned at a minimum distance of 3 m from neighbour plants of the opposing species to prevent potential confounding effects on soil chemistry of neighbouring plots. The plots were weeded as necessary to exclude influences of all other plant species.

Plate 5.2 A single species plot of medium sized (about 1.80m) *B. davidii* in midwinter in the Puhi-Puhi river.

Plate 5.3 A typical mixed plot in early spring with bigger plants (2.80m height for buddleia) in a medium-dense surrounding vegetation of mostly *C. arborea* in the Hapuku river.

5.2.3 Plant growth and assessment of leaf- and soil nutrients

Measurements of height, canopy diameter and basal diameter (5cm above ground level) on all plants were taken in August 2005 (winter) and in May 2006 (autumn). The increment in plant size (height, canopy area (calculated as an ellipse), and basal area) over the ten month growing period was calculated for individual plants. Aboveground biomass was calculated for each measurement using allometric relationships. The following relationships were best predictors for biomass development for a size range of up to 5.5m height and 17.5m^2 crown area (buddleia, n = 19) and 5.5m height and 345cm^2 basal area (coriaria, n = 18):

Biomass = 491.9 ^{x} height ^ 0.0235 ^{x} crown area ^ 0.7287 (r^2 = 0.87) -buddleia,

Biomass = 88.35 ^{x} height ^ 1.8065 ^{x} basal area ^ 0.7239 (r^2 = 0.99) -coriaria.

In November 2005 (spring), a minimum of 10 fully expanded canopy leaves of each plant were collected for analyses of foliar nitrogen and phosphate. Samples were dried (70^{o}C until constant mass was reached), weighed and ground. The total N content and the ^{15}N abundance in leaves were determined by isotope ratio mass spectroscopy using a CNS autoanalyser (Carlo Erba NCS 2500, Milan, Italy). Samples for P concentration were acid-digested using a Kjeldahl method and analysed colorimetrically (Blakemore *et al.*, 1987).

For the assessment of available soil N (NH_4^+ and NO_3^-) and P (PO_4^{3-}) during spring resin bags (5g of moist Dowex® Mixed Bed Resin, MR-3, Sigma Aldrich Australia enclosed in 5cm^2 Swiss Screens PE 48GG -365 µm polyethylene netting) were buried in the top layer of the soil (10-15cm depth) in about 10cm vicinity of the main stem of each plant (in mixed plots between both target plants) and left *in situ* for three months. The resin was eluted five times with 20ml 2M KCL to give 100ml extract (Hubner *et al.*, 1991) of which 1ml was used in the colorimetric analysis of soil ions. NO^{3-} was analysed using the cadmium- reduction method (Huffman and Barbarick, 1981; Stewart *et al.*, 1993.), NH_4^+ using the polyphenol method (McCullough, 1967) and PO_4^{3-} using the molybdate tartrate method (Solorzano and Sharp, 1980).

5.2.4 Statistical analysis

All variables were tested for normality and homogeneity of variance. Crown area was log - transformed prior to performing the statistical tests. Flooding events did occur in the study period, but damaged plots (5 of the originally 53 plots) have not been included in the analysis. Nested and mixed models with terrace as a random effect were applied to the data but the small sample size (n = 6 per treatment within rivers) and significant differences between rivers and terraces obscured treatment differences between treatments. To increase the statistical power, a GLM was applied to the pooled data (n = 12 per treatment) including

river and treatment as class variables to analyse treatment differences in soil and leaf ion concentration and plant growth. Differences in diameter growth increment and biomass production were modelled as a function of initial values. The influences of neighbour density added as a categorical variable (estimated biomass of the vegetation in 2 m diameter from the target plant) on those variables was analysed using GLM. Data analysis was undertaken using SAS, version 9.1 (SAS Institute, 1996).

5.3 Results

5.3.1 Plant growth and species interaction

Plant growth varied greatly with standard errors reaching up to 42% of the average value in buddleia (crown area increment) and 25% in coriaria (biomass increment). For all measured variables, plants of both species were slightly larger (on average 12% for buddleia and 18% for coriaria) in the mixed species plots than those in single species plots (Table 5.1), however, none of the observed differences between treatments were statistically significant at $P < 0.05$. The increment in growth over the eight month growing period for buddleia was also higher in mixed plots, ranging from 49-67% (Table 5.2) when compared to plants on single species plots; similarly, biomass production was 46% higher in mixed plots. Treatment differences between mixed and single species plots for buddleia were marginally significant for height ($P = 0.083$) only. For coriaria, the increment in crown area and basal area in coriaria was greater (51 and 9%, respectively) in plants growing on mixed plots, but the increment in height was smaller (20%). Therefore, for coriaria no treatment difference in the biomass increment derived from height and basal area was found. In summary, the growth of both species and the biomass production of buddleia tended to be higher in

mixture than in monoculture, but these effects were not statistically significant. Differences between terraces influenced plant performance more than initial size or treatment.

Table 5.1 The absolute difference (Diff) in plant size for buddleia (B) and coriaria (C) in monospecific and mixed (mix) species plots in August 2005 (initial) and May 2006 (final values). Values represent the average ± SE (*n* = 12).

	Height (m)		Crown area (m^2)		Basal area (cm^2)		Aboveground biomass (kg)	
	August	May	August	May	August	May	August	May
B	1.83 ± 0.08	2.20 ± 0.15	5.80 ± 1.09	9.00 ± 1.50	10.2 ± 1.94	16.7 ± 2.58	1.72 ± 0.24	2.41 ± 0.30
B mix	1.87 ± 0.10	2.43 ± 0.10	5.90 ± 0.90	10.6 ± 1.9	11.9 ± 2.06	22.1 ± 4.48	1.77 ± 0.21	2.72 ± 0.36
Diff (%)	2.4	10.4	1.8	18.3	16.6	32.2	2.9	12.6
C	1.38 ± 0.09	2.78 ± 0.16	16.2 ± 2.36	28.9 ± 3.38	43.4 ± 6.37	94.2 ± 12.2	2.60 ± 0.50	15.6 ± 2.14
C mix	1.54 ± 0.17	2.66 ± 0.22	18.8 ± 1.81	38.1 ± 4.60	51.6 ± 5.78	106.8 ± 14.9	3.92 ± 0.83	17.0 ± 3.54
Diff (%)	11.8	-4.3	16.4	31.8	18.9	13.3	50.3	9.1

Table 5.2 The difference (Diff) in the absolute growth increment of buddleia (B) and coriaria (C) - plants grown in monospecific and mixed species plots (B mix, C mix) over one growing season. Values represent the average ± SE (n = 12). P-values from ANOVA show that differences between treatments were not significant at $P < 0.05$ while the location of the plots (terrace) was of great importance.

	Height (m)	Crown area (m^2)	Basal area (cm^2)	Aboveground biomass (kg)
B	0.38 ± 0.10	3.00 ± 1.33	6.11 ± 1.79	0.65 ± 0.26
B mix	0.56 ± 0.09	4.74 ± 1.27	10.2 ± 2.91	0.95 ± 0.23
Diff (%)	49.4	58.1	66.6	45.5
P-value				
Initial	0.885	0.458	0.0076	0.845
Terrace	0.001	0.011	0.0008	0.007
Treatment	0.083	0.387	0.2368	0.444
C	1.40 ± 0.12	12.7 ± 2.16	50.8 ± 6.27	13.0 ± 1.81
C mix	1.12 ± 0.22	19.2 ± 4.36	55.2 ± 11.2	13.1 ± 3.34
Diff (%)	-20.2	51.3	8.6	0.9
P-value				
Initial	0.004	0.752	0.0129	0.3889
Terrace	0.000	0.686	0.0181	0.0003
Treatment	0.973	0.330	0.7287	0.7586

5.3.2 Available soil phosphorus and nitrogen during spring

Soil nutrient availability of the tested ions was consistently lowest under buddleia monocultures (Table 5.3). Interestingly, the availability of PO_4^{3-} and NH_4^+ under buddleia was almost as low as under control plots (bare soil). The availability of NO_3^-, however, was significantly lower under buddleia compared with the control. Mixed species plots showed intermediate amounts of all measured ions, while the highest availability of all soil ions was found in coriaria plots. In the latter, the amount of available PO_4^{3-} was about twice that under buddleia or bare soil (control) while amounts of NO_3^- and NH_4^+ were 3 and 4-fold higher, respectively.

Table 5.3 The available amount of soil ions over the spring period (Aug-Nov) 2005 shown as mean nutrient availability ± SE (n = 12) from the Hapuku and Puhi-Puhi rivers. Treatment differences indicated are significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

	PO_4 (µmol g Resin^{-1})	NO_3 (mmol g Resin^{-1})	NH_4 (mmol g Resin^{-1})
B	23.6 ± 3.6	1.3 ± 0.5	1.3 ± 0.3
C	40.2 ± 7.4	9.5 ± 3.2	5.1 ± 1.2
Mix	29.5 ± 4.3	3.5 ± 1.6	3.9 ± 1.3
Soil	22.2 ± 5.2	3.3 ± 1.3	1.2 ± 0.2
Difference between plots (%)			
B/ Mix	25.3	157.7	205.8 *
C/ Mix	-26.6	-63.4 *	-23.7
Soil/ B	6.0	-59.1 **	4.8
Soil/ C	80.9 *	188.5	320.1 *

The input of PO_4^{3-} and NH_4^+ under coriaria was clearly significant during the spring. Thus, for buddleia in mixed plots the availability of all measured ions was considerably greater compared to buddleia monoculture plots. For NH_4^+ this was significant at $P < 0.02$. In contrast, for coriaria, all measured soil ions were less available in mixed plots in comparison to single species plots during spring. This was significant for NO_3^- at $P < 0.01$.

5.3.3 Leaf phosphorus and nitrogen concentration

The concentration of leaf nitrogen and phosphate in November reflects the soil nutrient availability during spring (Table 5.4).The total nitrogen concentration was highest in coriaria plants when growing without buddleia, lowest in buddleia when growing alone and intermediate in both species when growing together. The highest values for total phosphate were observed in buddleia plants growing on mixed plots, closely followed by coriaria plants growing alone. Thus, values for total nitrogen and phosphate in buddleia were significantly higher (22 and 19%, respectively) when plants were growing in close proximity with coriaria in contrast to growing alone. In comparison, leaf nitrogen and P for coriaria growing in mixed plots were lower (7 and 11%, respectively). The parameter $\delta\ ^{15}N$ is the signature of ^{15}N in a sample and indicates how much the sample is enriched or depleted in ^{15}N relative to a reference. In the case of N the standard is air with a $\delta\ ^{15}N$ of 0 % by definition. $\delta\ ^{15}N$ is useful in distinguishing, for example, which N source a plant (or animal) is accessing ($\delta\ ^{15}N$ of nitrogen fixing species is usually close to zero, as they derive most of their N from the atmosphere) or the level of fractionation that has occurred during the uptake and incorporation of N (the heavier ^{15}N accumulates in the residue while products are depleted because it is slower to react than ^{14}N). When single and mixed species plots are compared, the signature $\delta^{15}N$ in coriaria growing on mixed plots was significantly closer to zero while values for buddleia of both plot types were not significantly different. In summary, these

data suggest that buddleia benefits nutritionally foliar nutrient concentrations in the presence of coriaria, but not vice versa.

Table 5.4 Mean leaf nutrient concentration and $\delta^{15}N$ ± SE (n = 12) in November 2005 (spring). Treatment differences significant at $P < 0.05$ are indicated by an asterisk.

	Total P (%)	$\delta^{15}N$ rel. Air (‰)	Total N (%)
B	0.13 ± 0.01	0.35 ± 0.5	1.6 ± 0.2
B mix	0.15 ± 0.01	0.57 ± 0.5	1.9 ± 0.2
C	0.14 ± 0.01	-1.1 ± 0.15	2.4 ± 0.06
C mix	0.12 ± 0.01	-0.03 ± 0.5	2.2 ± 0.1
Treatment difference (%)			
B/ Mix	19.0 *	-	22.4 *
C/ Mix	-10.9	*	-6.6

5.4 Discussion

5.4.1 Plant growth and species interaction

This study shows that both buddleia and coriaria were larger when growing together compared to growing alone, indicating that both species seem to benefit from their co-existence in river beds. While the effect size (i.e., the change in biomass or growth) was large (i.e., a 46% greater increment in biomass for *B. davidii* grown in close proximity to *C. arborea* compared to plants grown in monoculture) the power of the statistical test was low because of the low replication and high variability in plant growth (n = 6 per treatment and

river). No treatment differences in the measured growth variables were significant, and therefore this study would agree with Bellingham *et al.* (2005) who found no facilitative effects between the same species over successional seres spanning > 20 yr. However, the greater increment in buddleia biomass in the mixed plots indicate that there may be a trend of facilitation from the native nitrogen fixer for growth of this invasive shrub which would require further investigations over a longer time span and with more replicates. Despite a greater increment in crown area, the increment in biomass in *C. arborea* was similar in monocultures and mixed plots, so that no facilitative effect from the invasive *B. davidii* for growth of *C. arborea* was observed. However, biomass estimates for *C. arborea* were partially derived from height (best fit; $r^2 = 0.99$), but underestimation of biomass may still be possible, because the long, heavy branches of coriaria tended to bend down with increasing length (indicated by increased crown area). The biggest effect observed was the significant difference in plant growth between terraces. This suggests that strong site effects may overwhelm neighbour effects and that species interaction varies with the physical environment.

In contrast to Bellingham *et al.* (2005), the highest availability of all tested soil ions was found in soils under coriaria and not under mixed plots (Table 5.3), where litter input from both species should support the highest mineralisation rates. High litter accumulation and mineralisation under the wide canopies of coriaria together with additional input from nitrogen fixation (Silvester *et al.*, 1979; Clarkson and Clarkson, 1995; Walker *et al.*, 2003) may be responsible for the significant input of NH_4^+ (twofold) and PO_4^{3-} (fourfold) under coriaria monocultures compared to bare soil while in mixed stands the additional soil uptake from buddleia may be responsible for a decrease of soil ion availability to intermediate levels. Walker *et al.* (2003) found an even greater increase in soil fertility (tenfold in NH_4^+)

under coriaria, which resulted in facilitation of a native *Griselinia littoralis*. Such facilitative effects would likely explain the increased growth of buddleia when growing near coriaria.

The mass-ratio theory proposes that ecosystem properties are controlled to an overwhelming extent by the traits and functions of the dominant species (plants that are larger and contribute most to standing biomass in the vegetation) while transitory species (species of low abundance and persistence) are less influential (Grime, 1998). This seems to be true for the system studied here. Coriaria plants were not only much larger but also gained much more in biomass (13.0 ± 0.05 kg) than buddleia plants (0.8 ± 0.14 kg) over the ten-month growth period, which would agree with their much greater influence on soil chemistry compared to buddleia. However, competition for above ground resources (for instance, light) between neighbours is often size asymmetric. This means that the larger neighbour gets a disproportionate share of resources relative to its size because of pre-emptive resource depletion, and may therefore suppress the growth of smaller neighbours (Schwinning and Weiner, 1998). This seems not to apply in this study. Although light interception was not measured, coriaria plants were much larger (for instance, 3.6 fold in crown area) compared to buddleia plants, but buddleia was not suppressed by coriaria. The results from this study show that over the spring period, significantly more N and P was available for buddleia in mixed stands in comparison to buddleia monocultures, which led to increased growth of buddleia in mixed stands. Here, the facilitative effects from additional soil nutrient availability negated any negative effects of competition from the vigorous neighbour coriaria. As competition and facilitation shift during succession the long-term effects remain unknown and need to be disentangled by using a more elaborate experimental design, long-term studies or space-for-time treatments.

Still, little is known about the mechanisms that modify the degree of competition. Here, not only the mechanisms of resource capture are important, but also variables like the

spatial distribution and expansion of individuals. Generally the degree of asymmetry in competition increases with plant density (Weiner, 1985; Shabel and Peart, 1994), but exceptions were found where plants in very poor soils (Newbery and Newman, 1978) reduced competition owing to a general smaller plant size and a greater distance between individuals. While in this system soil fertility was indeed poor, the plant density was still high (especially in mixed stands, where the coriaria and buddleia of reasonable size were growing often with intertwined stems, i.e. had emerged almost in the same spot) but no noticeable competition was observed between species.

Other factors are important that are not easy to measure, for instance the plasticity of plants (i.e. the ability of plants to adjust morphologically and physiologically to environmental pressures but also to competition from neighbours. Here a greater allocation to structures or features that alleviate competition from neighbours are thought to be of major importance (Weiner and Thomas, 1992), for examples, increasing height growth in dense stands (Weller, 1987) to increasing light capture by placing leaves above those of neighbours or the avoidance of overlapping crowns in trees by suppression of branch buds (Jones and Harper, 1987). It could be speculated that the high growth capacity (in regulating leaf area growth (Chapter 2) and height growth of stems to avoid shading, together with a high resource use efficiency observed by (Feng *et al.*, 2007; Watt *et al.*, 2007) may contribute to the alleviation of possible competition effects from coriaria.

The foliar nutrient concentration reflected the soil nutrient availability and species interaction effects. Buddleia plants growing with coriaria had higher foliar nutrient concentration in comparison to plants in buddleia monocultures (19 and 22% for total P and total N, respectively), which would indicate that the increased soil nutrient availability is effective and would explain the growth increment in buddleia. In contrast, foliar nutrient concentration of coriaria in mixtures was lower than in monocultures (11 and 7% for total P

and total N, respectively), reflecting lower soil nutrient availability, particularly of nitrate. Despite this, the biomass production of coriaria plants in these stands was undiminished. The significantly higher values of foliar $\delta^{15}N$ for coriaria plants growing in mixed stands suggest that direct input from symbiotic nitrogen fixation had increased to remedy the reduced availability of soil nitrogen caused presumably by competition from buddleia. This may partially explain why *C. arborea* is not quickly outcompeted by *B. davidii* as observed by (Smale, 1990). Furthermore, pioneer species like *C. arborea* are generally well adapted to a nutrient poor environment and may have a high nutrient-use efficiency. This was confirmed by dos Santos *et al.* (2006) for species of the genus *Cecropia* in contrast to later successional species. If this applies to *C. arborea*, a lowered phosphate or nitrogen supply from competition of buddleia need not necessarily have detrimental effects on growth capacity and may likely be negated by greater general nutrient availability during spring and summer, when higher temperatures will increase soil microbe activity. In summary, buddleia growth and foliar nutrient status increased in the presence of coriaria, but no similar benefits were observed for coriaria. Coriaria appeared to increase nutrient capture to compensate for buddleia uptake in mixtures.

5.4.2 Influence of *Buddleia davidii* on soil nutrient availability

The amount of available nitrogen and phosphate in soils under the invasive buddleia was not significantly different from bare soil and was lowest among all vegetated plots. This is surprising in that other studies have reported that *B. davidii* invasions generally increase soil phosphate (Matson, 1990; Bellingham *et al.*, 2005). Different reasons could be responsible for this observation. There is evidence for seasonal variation in foliar P of buddleia and thus a different effect of litter on soil nutrients could be expected. In the present study (in spring), foliar P was not unusually high, whereas Bellingham *et al.* (2005) reports higher values of

foliar P for all successional stages of buddleia (up to 0.19% in mature stages) measured in summer. Phosphate may be stored in woody tissue over the winter period and increase in foliage only slowly with increasing temperatures and rates of metabolism in spring, so that litter accumulating over winter may be low in foliar P and therefore may not cause increments in soil phosphate availability. Furthermore, litter under buddleia shrubs does not tend to accumulate. Especially in winter, when the plants are without foliage, litter is quickly washed away by frequent floodings from between the thin, upright stems or blown away by wind, even in dense stands (Plate 5.2), so that in floodplains, buddleia litter may not contribute much to soil nutrient availability. The much lower biomass of buddleia plants in this study (7.5 fold lower when compared with coriaria plants in May 2005) may also explain the small influence of buddleia plants on soil nutrient availability in comparison to coriaria plants, but the effect of plant density within the sample area needs to be included to allow such conclusions.

It is most likely, that an impact of buddleia on soil chemistry takes time to develop and may therefore depend on the successional stage of the vegetation (Plate 5.3 vs. 5.4). Bellingham *et al.* (2005) found that soil P was positively correlated with the biomass of buddleia. Where no difference in foliar P between buddleia and coriaria was observed in early successional stages, in mature stages (i.e., after > 15 yr of succession) foliar P in buddleia was twice as high as in coriaria. The plants used in the present study would represent the young and vigorous stage of growth as mentioned in Bellingham *et al.* (2005) and were also sampled in spring, which may account for the difference. It is possible that stronger soil and facilitative effects would be observed in more mature successional stages, where events of floodings are less frequent. High VA-mycorrhizal colonisation of buddleia roots (see Chapter 6) may be one mechanism to allow the invader to colonise P-depleted soils, but this does not result in a general elevation of foliar P. Thus, while the input of P

and N from the native *C. arborea* was considerable during spring, the presence of the invader *B. davidii* did not affect soil chemistry to the same extent as previously reported by Matson (1990) and Bellingham *et al.* (2005).

Plate 5.4 A later successional stage (*B. davidii* –left, *C. arborea* -right).

5.5 Summary

In primary successions of New Zealand's floodplains, no significant facilitation from the native nitrogen fixing *C. arborea* can be reported although elevated foliar nutrient concentration and increased growth of the invasive shrub *B. davidii* were evident. The native *C. arborea* experienced competition for soil nutrients from the invasive species, but compensated for the reduced nutrient availability by increasing nitrogen fixation. Thus, no

negative effects of buddleia invasion on the growth of *C. arborea*, its indigenous neighbour, were observed. However, its needs to be remembered that competition between neighbours may be influenced by biomass and that, in this case, buddleia was much smaller than coriaria. This shows that flexible adaptation of native plants may, to a certain extent, negate possible negative effects from invasive species.

The impact of the invasive *B. davidii* on soil chemistry was small in relation to that of the native nitrogen fixer *C. arborea*, so that no facilitative effect (i.e. providing additional phosphate to the native nitrogen fixer) from the invasive *B. davidii* was found. Therefore, no strong evidence of an overyielding effect (i.e. the reciprocal facilitation between the two species) was observed. However, longer-term studies are needed to reveal the full extent of an invaders' impact on soil chemistry or other ecosystem properties.

6

MYCCORHIZAL ASSOCIATION IN *BUDDLEIA DAVIDII* FRANCH.

6

MYCCORHIZAL ASSOCIATION IN *BUDDLEIA DAVIDII* FRANCH.

6.1 Introduction

Vesicular arbuscular mycorrhizae (VAM) are a very important functional part in the plant – soil interface (Smith and Read, 1997) and can be found associated with the vast majority of land plants (Fitter, 1985). While recent research focuses on the role of mycorrhizae in ecosystem functioning on a broader scale (Read, 1991; Leake *et al.*, 2004; Rillig, 2004), there are numerous ways in which mycorrhizae influence plants: they can increase plant resistance to drought (Ruiz-Lozano *et al.*, 2001), heavy metals (Vivas *et al.*, 2003) and pathogens (Azcon-Aguilar and Barea, 1997b; Fritz *et al.*, 2006), but their best documented effect is that of increasing plant nutrition (Landeweert *et al.*, 2001; Chen *et al.*, 2003; Van der Heijden *et al.*, 2003; Govindarajulu *et al.*, 2005). Mycorrhizae can access organic sources of nitrogen, phosphorus and carbon and thus increase the availability of nutrients (e.g. recalcitrant P) that are otherwise inaccessible for plants (Smith and Read, 1997; Read *et al.*, 2004). Mycorrhizae have also been shown to increase the efficiency of phosphate acquisition in plants by considerably increasing the influx of phosphate (Koide *et al.*, 1999) through exploitation of a much greater soil volume and also access of sources that are

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otherwise inavailable to plants (Fitter, 1985; Bolan, 1991; Landeweert *et al.*, 2001). This can be especially important in ecosystems that are nutrient deficient (Smith and Read, 1997; Read *et al.*, 2004) such as primary successions with very young substrate. Mycorrhizae–mediated increased nutrient supply often shows in higher foliar concentration (Tibbett and Sanders, 2002; Dickie *et al.*, 2007a). Despite a certain carbon cost, improvement in growth or other aspects of fitness is often evident in mycorrhizal plants in comparison to non-mycorrhizal plants, giving rise to the increased use of inoculation techniques in horticulture (Azcon-Aguilar and Barea, 1997a) and managed environments (Azcon-Aguilar *et al.*, 2003; Jeffries *et al.*, 2003). Mycorrhizae can be especially important during critical stages of plant life history like seedling establishment (Turnbull *et al.*, 1995; Dickie *et al.*, 2002; Dickie *et al.*, 2007a).

Very high foliar N and P concentrations have been reported for species of buddleia from different environments. For example, *B. asiatica* in primary successions on volcanic ash deposits in Hawaii showed a foliar N concentration twice as high (2.5 ± 0.14%) as the nitrogen fixer *Myrica faya* (1.34 ± 0.059%) and a foliar P concentration four-fold that of myrica (0.206 ± 0.015% vs. 0.052 ± 0.004%) and had thus the highest foliar N- and P concentration amongst all measured species (Matson, 1990). A comparison of the native nitrogen fixer *Coriaria arborea* and the invasive *B. davidii* on primary successions on flood plains in New Zealand revealed that, in the mature successional stages, buddleia reached values of foliar N similar to those of the nitrogen fixer and values of foliar P twice as high as that of coriaria (Bellingham *et al.*, 2005). To date, it is not clear, how buddleia plants acquire the unusually high foliar P levels that are frequently reported. A highly efficient uptake mechanism in roots or a mediator such as mycorrhizal association was hypothesised (Bellingham *et al.*, 2005). The mycorrhizal status of *B. davidii* is still unclear as the only two available reports (Stevenson, 1964; Harley and Harley, 1987) are contradictory. Since the

species is reported to have high impacts on soil chemistry and its considerable growth capacity and invasiveness, it is important to understand the mechanism of phosphorus acquisition. Here, the mycorrhizal status of *B. davidii* is investigated to explore possible explanations for high levels of foliar nutrients.

6.2 Methods

The study site was located on flood plain terraces of the Hapuku (42^o 18' S, 173^o 41' E, 128m a.s.l.) and Puhi-Puhi rivers (42^o 16' S, 173^o 43' E, 142m a.s.l.), on the eastern South Island of New Zealand as described in detail in Chapter 5 (Figure 5.1). In May 2006, samples of fine roots (<2mm in diameter) were taken from ten individual buddleia plants and three coriaria plants to investigate mycorrhizal infection in field grown plants. The plants chosen were of average size (about 1.5m in height) and were growing separately from other vegetation, i.e. as single individuals. Roots were excavated to a depth of 15cm and traced back to the plant of origin to ensure that the roots belong to that individual sample plant. Roots (fine roots to a diameter of 2mm) were cut and transferred in 70% ethanol from the field site. Root samples were then washed and cleared for 30 minutes in 10% KOH, rinsed in water, acidified with 5% HCL for 5 minutes, stained for at least 24 hours in 45% H_2O, 50% Glycerol, 5% acetic acid and 0.01% trypan-blue, and then embedded in 50/50 lacto-glycerol (Dickie *et al.*, 2001). The average percentage of VAM-infections of roots was estimated on 10 samples and counted as VAM -infected when both hyphae and arbuscules were seen.

6.3 Results

Vesicular-arbuscular mycorrhizae (VAM) in roots of field grown *B. davidii* plants were found (Plate 6.1a, b). On a total of 10 samples the mean rate of infection was 97.1 ± 1.2% of root length with extensive arbuscules (74.7 ± 7.2 %) and vesicles (38.6 ± 4.7 %). Roots of *C. arborea* was also heavily infected with VAM (hyphae 98.7 ± 0.9%, arbuscules 91.0 ± 4.0%, vesicles 74.0 ± 4.2%, $n = 3$; Plate 6.1 c).

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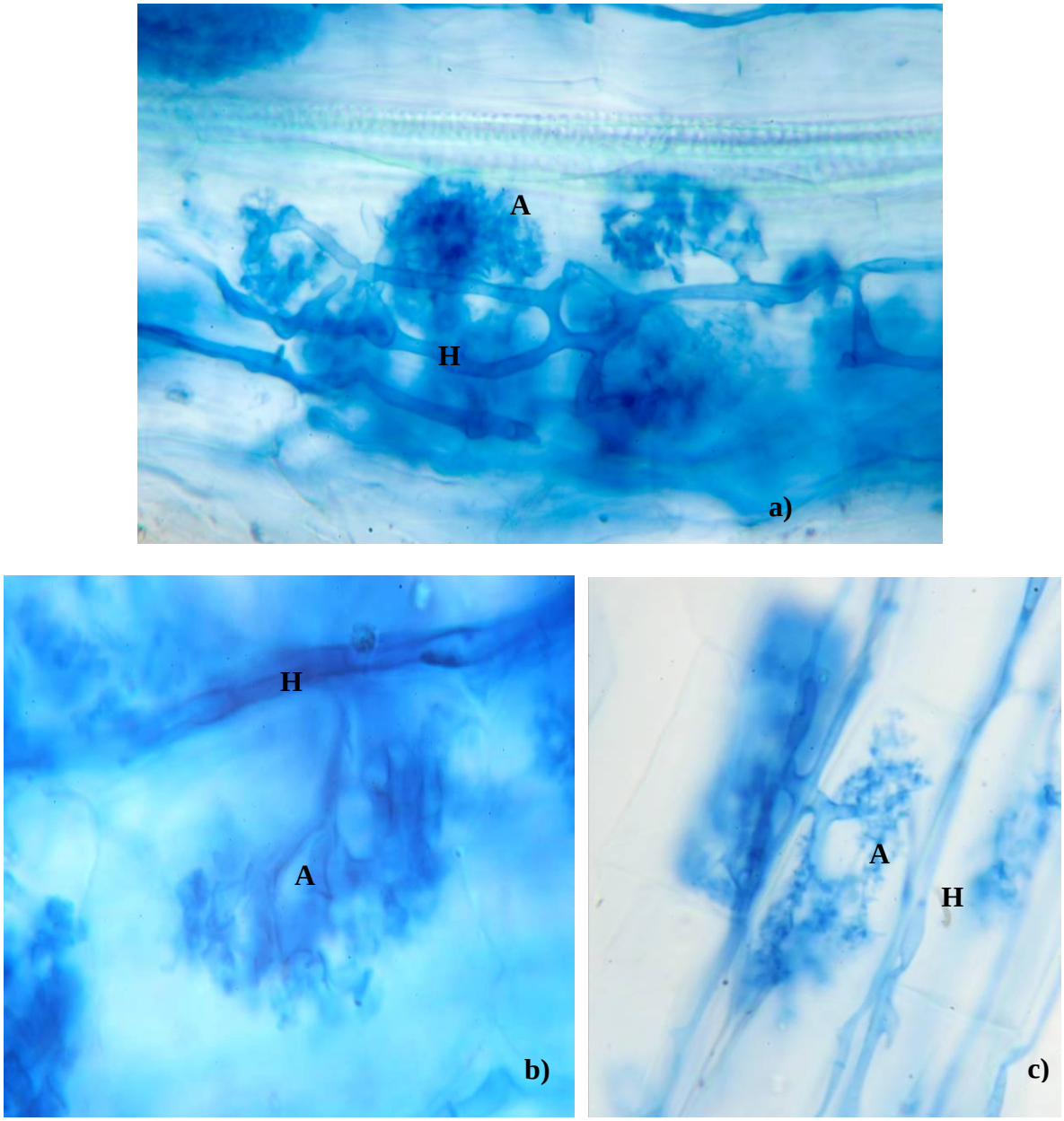

Plate 6.1 **a), b)** VAM infection in fine roots of *B. davidii* Franchet and **c)** on roots of *Coriaria arborea* Lindsey. The characteristic structures of VAM are arbuscules which extend from the fungal hyphae (H) and are visible as tree like structures (A) surrounding the protoplast.

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6.4 Discussion

The results that *B. davidii* roots are heavily colonised by VAM resolve the question of the mycorrhizal status of the *B. davidii* Franch. Only few observations are available on mycorrhizal colonisation in other species within the genus buddleia: Camargi-Ricalde *et al.* (2003) report low VAM-infection (up to 10% root length) in *Buddleia* spp in Mexico and Laughton (1964) reports hyphae in *Buddleia* [sic] *salviifolia* (L.) Lam. in South Africa, but without arbuscules. For *B. asiatica* Lour. in Hawaii, VA –mycorrhizae have been suggested by Matson (1990), which was supported by Koske *et al.* (1992) who found a root infection of 25 -75% in this species. For *B. davidii* Franch. only two publications are available one of which lists the species as non-mycorrhizal in Britain (Harley and Harley, 1987), while the other reports *B. davidii* as VAM positive (Stevenson, 1964). Naturalised *B. davidii* on primary floodplain successions in New Zealand examined in this study were extensively colonised (97%) by VA-mycorrhizae (Plate 6.1). Recently, high VAM infection (69%) was also found in root samples of *B. davidii* from the UK (Dickie *et al.*, 2007b) which strengthens the evidence that the species *B. davidii* Franch. is positive VA-mycorrhizal.

This extensive mycorrhizal colonisation of *B. davidii* is likely responsible for the high P concentration that is often found in leaves and in soils under buddleia (Matson, 1990; Bellingham *et al.*, 2005). Since mycorrhizae can also increase the pool of nitrogen available to plants by breaking down organic nitrogen sources (Turnbull *et al.*, 1995; Tibbett and Sanders, 2002), the mycorrhizal mediated increased availability in these two most important nutrients for plant growth, may partially be responsible for the invasive success of buddleia plants in primary successions on flood plains (Chapter 5). VA-mycorrhizae were also

observed on *C. arborea* which was expected and agrees with findings of Tiwari *et al.* (2003), who found arbuscular mycorrhizae and actinorrhizae on other species of Coriariaceae.

6.5 Further research

Recent research found evidence for a reciprocal nutrient transfer via fungal hyphae between co-occurring plants (He *et al.*, 2003; Carey *et al.*, 2004; He *et al.*, 2006). The amount transferred may be considerable and the importance of this mechanism in plant nutrition may be underestimated (Moyer-Henry *et al.*, 2006). For example, Carey *et al.* (2004) found evidence for mycorrhizae –mediated carbon transfer from a native to an invasive species, leading to increment in biomass despite reduced photosynthesis in the invasive species. This was thought to provide a competitive advantage for the invader and thus facilitate the displacement of native species by weeds. Such a mechanism may be especially important in primary succession, where nitrogen fixing and non-nitrogen fixing plants co-occur. More generally, fungi may play an important role in assisting weed invasions (Fitter, 2005) and if nutrient transfer or parasitism via hyphae was a general principle, a new dimension would be added to our understanding of the success of invasive species. However, to ascertain the magnitude and role of this mechanism in plant nutrition will require further investigation.

Buddleia infestations in natural environments may also have indirect effects by introduction of their associated mycorrhizae. Recently, the importance of the plant-soil feedback in shaping plant communities and ecosystem functioning has been recognised (Klironomos, 2002; Callaway *et al.*, 2004; Reinhart and Callaway, 2006). Especially, the effects of invaders belowground (i.e. that invasive plants may alter communities of soil biota

with subsequent impact on the performance of other plant species) are important, but previously poorly recognised (Van der Putten *et al.*, 2007). Ecosystems differ in their fungal communities which represent an evolutional selection of functional attributes best suited for that specific environment. Here, more and more interdependencies (for instance, trophic relationships between different partners) are being revealed at community level (Smith and Read, 1997; Wardle *et al.*, 2004). Trophic effects and interactions have recently been recognised as important drivers of invasion success and impacts in the ecological literature (Richardson *et al.*, 2000; Klironomos, 2002; Callaway *et al.*, 2004). Thus, changes in the composition of the mycorrhizal community induced by the introduction of invasive species (Mummey and Rillig, 2006), may have implications for the stability and composition of plant communities. Further investigation of the impacts of a foreign fungal species on plant communities may be of great relevance (Schwartz *et al.*, 2006).

6.6 Summary

The new evidence of VA –mycorrhizae in *B. davidii* resolves the controversy about the mycorrhizal status of the species and will likely be responsible for its ability to invade and thrive in nutrient poor soils. While the mycorrhizal association partially explains the invasive success of buddleia in these habitats, it did not generally lead to an increased foliar phosphate level, which is often reported but could not be confirmed in this study.

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7

GENERAL DISCUSSION

7

GENERAL DISCUSSION

This research was undertaken to investigate the growth response of the invasive shrub *B. davidii* to repeated high levels of defoliation designed to mimic a potential biocontrol. The project included detailed studies of the effect of defoliation on seasonal leaf area dynamics, photosynthesis, flower and seed production, germination and nitrogen translocation within tissues in potted plants in semi-controlled conditions. To address additional factors that are important to buddleia invasion in the natural environment, a field study was undertaken to investigate species interaction (i.e. possible facilitation of the weed from neighbouring nitrogen fixer plants) and the influence of *B. davidii* on soil chemistry. The questions raised in the conceptual model in the introductory chapter (Figure 1.1) are reassessed here (Figure 7.1) in the light of the results obtained from these investigations.

7.1 Leaf Area dynamics in *Buddleia davidii*

7.1.1 Compensation and regulation

Figure 7.1 - 1 illustrates the physiological processes involved in regulating the defoliation-induced compensational leaf area growth in *B. davidii* (letters in the figure may assist the

reader in following the main arguments in the text). Compensational growth after defoliation is common in plants (Vanderklein and Reich, 1999; Anten *et al.*, 2003; Parra-Tabla *et al.*, 2004; Newingham *et al.*, 2005) and can be viewed as a mechanism to counteract losses in carbon assimilation (a_{ii}) which provides the foundation for all plant growth and fitness. In *B. davidii* the main mechanism facilitating the compensatory leaf area production (b) was increased node development (c). Only through production of new meristems is it possible for a plant to put out new leaves, the characteristics (i.e. photosynthetic capacity (a_i), longevity (d) or leaf size (e)) of which can then be adjusted to meet the changed requirements.

On defoliated *B. davidii*, more nodes per shoot were produced (c) and an early release of dormant buds for the growth of new-season shoots and leaves was responsible for their higher leaf area over winter. While the node production still increased at the end of the experiment, the compensation in leaf area production that was observed in the short-term (f_i) was not sustained in the longer-term (f_{ii}). Despite an adequate supply of nutrients in this controlled experiment, the size of the newly grown leaves decreased (e) so that the compensation in leaf area production (b) declined again after only one growing season. The only other mechanism that counteracted the artificial leaf loss in the long term was the increased leaf longevity (d) of individual leaves. Similar to node production, leaf longevity increased to the end of the measurements to improve total carbon assimilation (a_{ii}) in the face of fewer and smaller leaves. No upregulation of the photosynthetic capacity (a_i) in pre-existing leaves was observed. However, to investigate the long term response of photosynthesis to defoliation in *B. davidii*, it would be instructive to also measure photosynthesis in new-grown leaves, which was not within the scope of this study.

This research revealed that plants may show great plasticity in response to manipulation and that *B. davidii* increased light capture primarily through adjustment of characteristics of leaves and shoot growth. The research also showed that the availability of

nutrients and tissue resources is vital in determining the magnitude and duration of any compensation in plants after defoliation. Here, improving of the cost efficiency of leaves by increasing their longevity (i.e. making efficient use of resources already available) was of great importance in facilitating compensation.

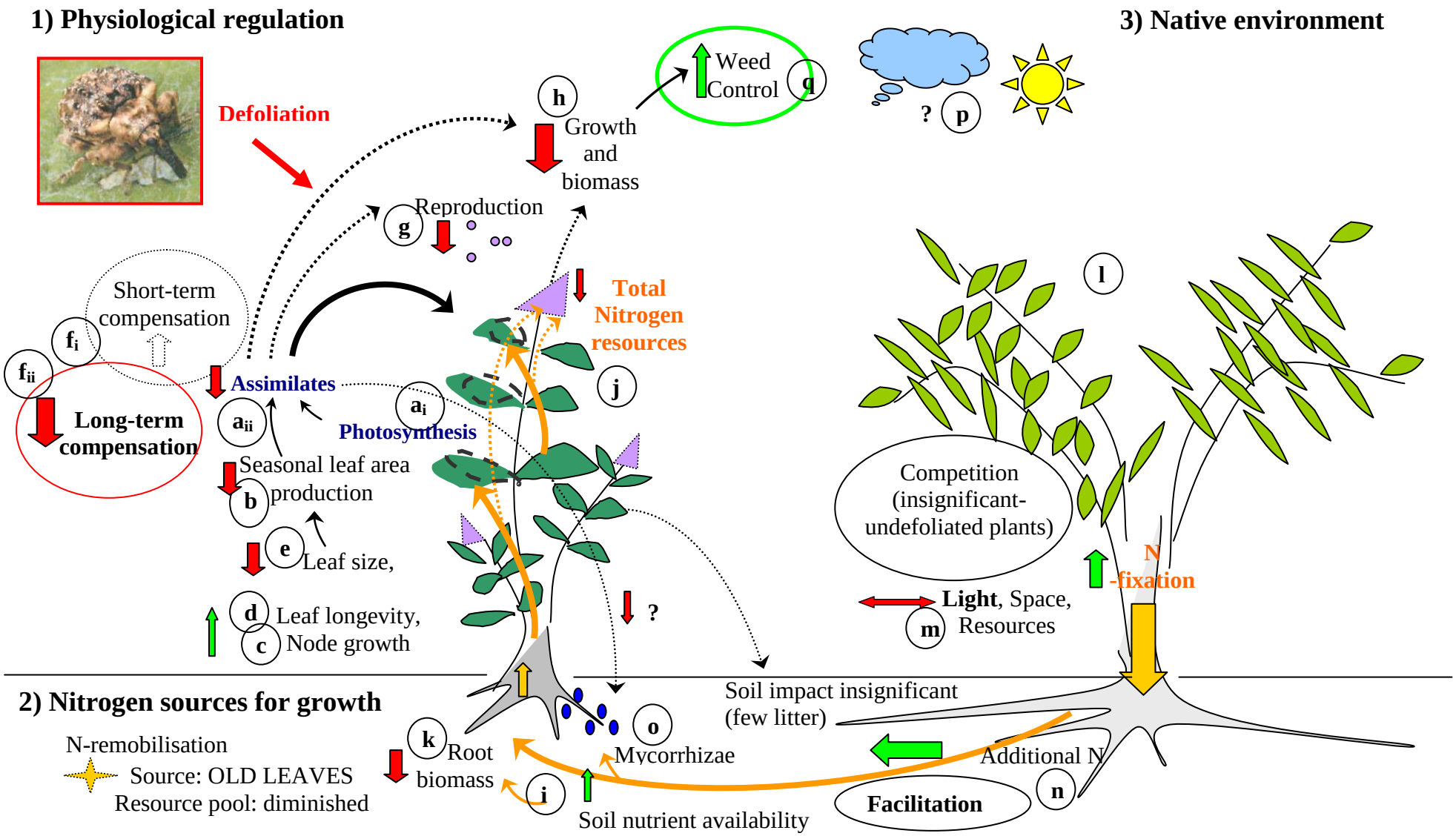

Figure 7.1 The physiological regulation of the response of *B. davidii* to defoliation and the relationship with its neighbour *C. arborea* in the field.

7.1.2 Trade–off between growth and reproduction

The ultimate goal for plant growth is to support reproduction (g), but flower and seed production require significant resources and are therefore very costly for plants (Banuelos and Obeso, 2004). As different sinks (b, g, h) compete for assimilates (a_{ii}), an increase in vegetative growth may therefore occur at the expense of reproductive output (Obeso, 1997; 2002; Kawamura and Takeda, 2006). Resources necessary for growth are supplied through root uptake of soil nutrients (i) and also through photosynthetic carbon assimilation by the leaves (a_i). Here a trade-off may be involved in defining the balance between resource allocation to leaf growth (b) and fruit production (g) in some species (Mendez and Obeso, 1993; Silvertown *et al.*, 1993; Reekle *et al.*, 1997).

Defoliation may severely decrease a plant's assimilatory capacity (a_i) which offsets this balance. The reproductive output (g) is generally reduced where resources are allocated to regrowth of leaves to assure sufficient carbon assimilation and thus, survival of the plant. However, the trade-off between reproduction and growth seems to depend on the growth form of plants (Obeso, 1993). For example, the annual herb *Abutilon theophrasti* grown at low density has been observed to compensate with increased percentage of fruit set after 75% defoliation (Mabry and Wayne, 1997), which may be explained by the fact that annuals have no need for storing tissue reserves in perennial organs for the next season. In contrast to this, perennials need "to plan their expenses more carefully" to survive stressful periods (e.g. winter) with enough provisions for the new seasons' growth and may therefore often prioritize resource allocation to leaf growth. Although there are exceptions (see Escos *et al.* (1996)), woody perennials mostly experience a reduction in reproduction after defoliation (Tolvanen *et al.*, 1993; Anten *et al.*, 2003; Thalmann *et al.*, 2003) which is now also confirmed for *B. davidii* in this study. The magnitude of the reduction in seed production (g) in buddleia after only one season of defoliation was considerable (42%) and would help to

limit the reestablishment of this invasive species in the natural environment. With continued high levels of defoliation which will further reduce the assimilate supply to flower- and seed production, an impact on seed quality (for instance, a decrease in germination capacity or a reduced fitness of seedlings) may also be expected. As the impact of defoliation on reproduction will also depend on soil fertility (i) in the natural environment which buddleia invades, the investigation of the relationship between defoliation and reproduction at different levels of soil fertility would be a fruitful topic for further study.

7.2 Nitrogen – the foundation for growth

Compensational growth requires additional resources (Fig. 7.1-2). These can be supplied from root uptake (i) and/or from tissue resources within the plant (j). Nitrogen is of central importance for plant growth and is often the main limiting nutrient (Lundell, 2001; Vitousek *et al.*, 2002b). Tissue resources are of major importance where the soil supply is insufficient, for instance in spring (Millard and Proe, 1991; Bausenwein *et al.*, 2001), where temperatures are still too low to allow for adequate root uptake, or where plants grow on infertile soils (Millard and Neilsen, 1989). In New Zealand, buddleia primarily invades poor soils and has become a dominant species of primary successions on flood plains in rivers (Smale, 1990; Bellingham *et al.*, 2005).

The discovery that *B. davidii* stores nitrogen mainly in leaves is very important, because it strengthens the notion that biocontrol with insect herbivores will be very effective. Defoliation reduces leaf biomass, which will not only impair the plant's assimilatory capacity (a_{ii}) but also diminish the pool of tissue nitrogen for potential remobilisation (j). This study clearly shows that defoliation caused a major decline in tissue nitrogen resources

(j) with time, so that the amount of nitrogen that could be remobilised from storage was smaller. In defoliated buddleia old branches also contributed to nitrogen remobilisation in order to compensate for the reduced amount of nitrogen remobilised from old leaves, but since woody tissue contained only small amounts of nitrogen in comparison to leaves, this could not satisfy demands. This reduction in tissue nitrogen resources lead to an increased reliance of defoliated plants on soil uptake (i), but where plants grow on infertile soils, a greater root mass would be required to increase the uptake of nitrogen from the soil. However, the defoliation-induced reduction in assimilates together with an increased allocation of resources to new leaf growth caused a major decline in the production of root biomass (k), so that a reduced maximum uptake capacity would be expected. Since nitrogen is often the most limiting nutrient for plant growth, it is likely that, apart from reduced carbon assimilation at the plant level (a_{ii}), limitations in the nitrogen supply (j) would be another major contributor in reducing the compensational leaf area production with time (b). As it is unlikely that plants with reduced root growth will be able to replenish their nitrogen stores (j), especially so if they grow on poor soils, the success of biocontrol is likely to increase with time if high levels of defoliation continue.

In a review Galway *et al.* (2003) evaluated the performance of different feeder guilds on stressed and non-stressed plants and it appeared that leaf feeders responded positively to stressed plants,. The reason for their increased performance may be that stress in plants (reduced moisture, light, CO_2) is generally associated with increased foliar nitrogen which the feeders prefer. The same may then apply where younger plants are used or soil N is increased. But the relationships are too complex to allow simple predictions and it was cautioned to consider plant stress type and insect species. In defoliated buddleia a temporary increase in foliar N (accompanying compensational leaf growth) may be followed by a

decline as root uptake may be impaired by declining root growth. I am not aware of any feeding preferences of *C. japonicus*, but this *may influence the weevils feeding behaviour*.

7.3 *Buddleia davidii* invasions in natural environments

While it is relatively easy to predict the impact of defoliation on potted plants growing under defined and sheltered conditions, many more variables need to be considered concerning plants growing in the field (Fig. 7.1 -3). There are two factors that are of major of interest: nutrient supply (i) and species interactions (l). From what has been learned in this study, one might predict that defoliated plants growing in areas with higher nutrient availability (such as disturbed urban areas) would show greater compensation after leaf damage or may maintain compensation over longer time periods while plants that invade infertile habitats (such as river beds with frequent flooding and very young substrate) may experience greater stress and may thus be controlled more quickly. However, interactions with neighbours (l) (i.e. competitive (m) and facilitative effects (n)) add to the complexity of the situation in the natural environment and need to be evaluated. The magnitude of the influence from species interactions depend on the species involved and is particular important where nitrogen fixing species are involved.

The field experiment presented in this research indicated facilitation may be possible through additional provision of soil nitrogen (n) to *B. davidii* from the neighbouring nitrogen fixer *C. arborea*. Despite the fact that the treatment differences were not significant due to small sample sizes, the effect size was noticeable. Undefoliated buddleia plants growing near coriaria gained 46% more in biomass in one year compared with buddleia growing in monocultures. This was due to an increased availability of soil N (especially in

NH_4^+) and P from the presence of coriaria, mirrored also as elevated foliar nutrient concentration in buddleia plants growing near the nitrogen fixer. No negative effects of competition from coriaria were observed on buddleia in the field. However, since this study used undefoliated buddleia plants, it remains to be seen whether the additional stress of leaf damage from insect herbivores would outweigh the benefits from the surplus in nitrogen availability provided by coriaria. While the native nitrogen fixer *C. arborea* greatly increased both soil N and P availability, the influence of the invasive *B. davidii* was negligible. This contrasts findings of other studies which have reported an increased availability of phosphate in soils under buddleia. This study highlights that seasonal variation in soil nutrient availability can be high (here measured in spring as opposed to summer in other studies) and that the environment (soils prone to heavy flooding) and the successional stage of the vegetation (a correlation of soil phosphate with buddleia biomass was noted in mature stages, but younger stages were used in the present study) determine the impact of buddleia on soil nutrient availability. Therefore, generalisations of a high impact of buddleia on soil nutrient availability cannot be made.

Symbiosis with mycorrhizae (o) generally increases the uptake of nutrients in plants (Smith and Read, 1997; Tibbett and Sanders, 2002; Read *et al.*, 2004). In this study, it was confirmed that *B. davidii* is associated with VA-mycorrhizae. Such associations may enable the plants to colonise infertile environments but could also provide defoliated plants with the additional nutrients (i) required for compensational growth. However, plants allocate a substantial amount of carbohydrates to the fungus (Leake *et al.*, 2004) and it is not likely that defoliated plants, which produce fewer photoassimilates (a_{ii}), would be able to increase the carbohydrate flow to the fungus in order to increase its growth and hence provision of additional nutrients. Generally, defoliation reduces the host's investment in mycorrhizae which then reduces the percentage of root colonisation, as shown for *Betula pubescens*

(Markkola *et al.*, 2004). However, in defoliated *Pinus sylvestris*, changes occurred only in diversity and composition of morphotypes of ectomycorrhizal symbionts while the extent of colonisation remained high despite decreased sugar content in roots (Saikkonen *et al.*, 1999; Kuikka *et al.*, 2003). Therefore, further research would be required to investigate whether the mycorrhizae on buddleia plants are negatively affected by the defoliation-induced restrictions of carbohydrate supply (a_{ii}) from their host plant. A decline in mycorrhizal colonisation on defoliated plants may in turn result in an increased vulnerability in such plants, so that other environmental stressors (p), like high temperatures or drought, and competition (m) from neighbouring plants may gain in importance and override the positive influence of increased nitrogen availability from coriaria (n).

7.4 Prospects for biocontrol with leaf herbivores

The overall conclusion from this study is that the prospects for biocontrol (q) of *B. davidii* are very good. The damage (b, h, g) caused by artificial defoliation on potted plants was considerable. A simple extrapolation of the results would indicate that, given a scenario where the treatment continues at the same level (66% defoliation) and under the same conditions (adequate nutrition and water), an individual plant should stop producing seeds after two years and will cease growing and producing leaves and, therefore, die after four years. This, of course, does not incorporate any variation in the population dynamics of either plants or insects or the impacts of natural environment, and can therefore only serve as a guideline.

A first evaluation of the insect herbivore *Cleopus japonicus*, which was released in New Zealand in 2006, showed a damage intensity of about 60% defoliation on plants within the release area. While this is a promising start, it is yet too early to say with confidence if

the insects will effectively establish in New Zealand and what level of damage the weevils will achieve (M. Watson, Ensis Forest Biosecurity and Protection, New Zealand, personal communication, June 2007). The establishment of the insect agents depends on the climate of the new environment. However, the native range of *C. japonicus*, which is the same as that of *B. davidii* (S and SW China), experiences harsh climatic extremes in summer and in winter (N. Kay 1991 in ERMA (2005)), so that establishment in New Zealand's climate should not be problematic.

It is not clear whether the response of buddleia plants to the damage from insect agents under field conditions will be the same as that to artificial defoliation in a controlled experiment. In defoliation experiments, usually whole leaves are removed which causes an instantaneous drop in the assimilatory capacity of the plants and constitutes a major stimulus to provoke a strong compensatory response. In nature, the damage inflicted by insects is different. Defoliation of plants may be slower because the level of the damage depends on the population density and the appetite of the insects. For instance, if the damage is small (i.e. a part of the leaf remains undamaged and the leaf is not abscised), the reduction in assimilatory capacity and the overall impact on plant growth may be negligible in the light of the high growth capacity of *B. davidii*. A rather mild stimulus like this may allow plants the time necessary to adjust and respond to the herbivory, for instance, by producing chemical deterrents (Bennett and Wallsgrove, 1994; Zagrobelny *et al.*, 2004) which may then further reduce the impact of the biocontrol agents. Alternatively, if the population density of the insects is high enough and the damage severe enough, whole leaves may be discarded (Plate 7.1). This would have a similar impact to that of artificial defoliation. Regular reassessment of insect establishment and leaf damage to target plants is necessary in order to predict whether the plant response will be similar to that seen in studies employing artificial defoliation under semi-controlled conditions.

Plate 7.1 The weevil *Cleopus japonicus* Wingelmueller (larvae left, adult right) released in 2006 as a biocontrol agent for *B. davidii* in New Zealand. These weevils graze the upper green leaf surface, leaving a "window" of the lower epidermis. The damage will cause the leaf to dry out and eventually being abscised. Growth suppression from the damage will depend on the population density of the insect (Courtesy of ENSIS, Forest Biosecurity and Protection).

This study revealed that in *B. davidii*, the main facilitator for the production of new leaf area was increased node production. While this reflects the high growth capacity of this species, it also indicates possible benefits of introducing a second insect agent that specifically attacks shoot tips leading to stunted growth and thus prevents the development of new nodes. This option has been explored with investigations of the long leg weevil *Mecysolobus erro* (Kay *et al.*, 2003) and may be pursued further once actual figures of the success of *C. japonicus* are available (B. Richardson, Ensis Forest Biosecurity and Protection, New Zealand, personal communication, June 2007). Another very promising step would be the introduction of an insect to damage seeds. Although seed production declined significantly after defoliation, the strong germination potential of the remaining seeds (which are still produced in considerable quantity) will prolong the length of control efforts to be made.

7.5 Conclusion

This research successfully revealed the mechanisms involved in regulating compensational leaf area growth in response to defoliation in the invasive shrub *B. davidii* Franch. The main compensation of *B. davidii* after defoliation was to increase light capture. Through increased node production and early release of dormant buds in winter a higher leaf area was achieved as new grown leaves had increased in size and longevity. New insights into the general plant responses to artificial leaf loss are also provided by highlighting interesting differences in the response of individual shoot types to defoliation. As different shoot types are grown in a consecutive order during the seasons, differences in growth and performance between them can yield information about temporal changes in the compensational response. This can be indicative of the extent of the impact of defoliation on the plants resources and can therefore explain differences in the vigour of the compensational response of plants to defoliation treatment. This illustrates the importance of including different hierarchical levels in the investigation for a better understanding of the whole plant response.

Another very important variable influencing compensation in buddleia was nitrogen supply. The considerable defoliation-induced regrowth of leaf tissue to restore carbon assimilation capacity required additional nitrogen, but as old leaves were identified as the tissue of nitrogen storage, the pool of tissue nitrogen also declined greatly with leaf removal. The considerable reduction in nitrogen and assimilate supply caused a weakening in the compensational growth along with a considerable decline in biomass and seed production. Most important here was the great reduction in root mass, which will impair future soil uptake of nutrients and thus, also the replenishing of nitrogen stores. Thus, the responses of leaf phenology and nitrogen translocation clearly emphasize the importance of maintaining

high levels of defoliation for long durations of time to achieve control of growth and spread of the invasive shrub *B. davidii*.

This study also included a first exploration of how environmental parameters, especially the soil nutrient availability and species interaction with native neighbours, might influence the growth of *B. davidii* in the field. While no negative effects from competition with the native *C. arborea* were observed, enhanced soil nutrient availability from the nitrogen fixer, especially nitrogen, slightly increased biomass production of the invader. Also, this study provided new evidence for strong mycorrhizal colonisation of *B. davidii* in New Zealand (thus, rectifying contradictory reports on the mycorrhizal status of this species), which may be partly responsible for the invader's ability to colonise and thrive in infertile habitats. The field study of this work did not include defoliation treatment and only assessed nutrient availability and growth of buddleia in their natural surroundings. However, nutrient supply will certainly influence compensation after defoliation, so that it would be expected that, where buddleia invades nutrient poor sites, biocontrol may be more effective, while buddleia invasions in forest plantations may be more difficult to control. However, additional nutrients from fertilisation in those plantations that may increase buddleia growth would be counteracted by increasing competition from the *Pinus radiata* (a fast growing species commonly planted in New Zealand) both for nutrients and for light, which may reduce buddleia growth, because buddleia does not tolerate shading well. A complete investigation of all environmental parameters that might influence the impact of insect herbivores on *B. davidii* was outside the scope of this thesis. Here, the focus was to investigate the details of physiological responses, especially leaf area dynamics, to defoliation treatment.

Finally, this research has raised opportunities for further research which will be valuable for (i) predicting the success of weed control in this particular species, but also for

(ii) better understanding of the response of plants to defoliation and (iii) the impact of weeds on natural environments. Many exciting questions still need to be explored, for instance whether or not new grown leaves up-regulate photosynthesis in response to defoliation. It could be speculated that, these younger leaves (as opposed to pre existing leaves; Chapter 2) may show a greater plasticity and therefore may adjust their photosynthetic capacity in response to changing assimilate demands. This may be especially important where defoliation (at higher levels or after prolonged time periods) causes a decline in photoassimilates to a critical limit. Since the amount of stored nitrogen is considerably reduced by defoliation and thus the nitrogen uptake from the soil increases in importance (Chapter 4), defoliation treatment could be carried out at different levels of soil nitrogen supply to investigate the possibility of increased uptake capacity in roots after defoliation. This may be particularly important, given that in the field possible facilitation from nitrogen fixers enriches the soil with nitrogen. The effects of defoliation on mycorrhizal colonisation of buddleia may also be of interest, as in field-grown plants mycorrhizae-mediated nutrient uptake may constitute a considerable proportion of the plant's nutrient supply. Mycorrhizae can be very important in facilitating plant growth in infertile habitats in that they increase the availability of nutrients to the plants (Chapter 6), but their growth and performance depends on the provision of carbohydrate from the plant host. Since defoliation reduces the photosynthetic capacity of the target plant, the sugar supply to the fungus may also decline which may feedback to the performance of the plant. Concerning the impact of weed plants in natural environments, further study could explore if and how facilitation and competition from *C. arborea* in the field depends on plant density and successional stage of the vegetation. There is also a need to investigate if the impact of buddleia on soil chemistry during summer and autumn is similarly in natural systems. These questions are pertinent and

topical as only a limited number of studies on *B. davidii* are available to date, despite the fact that this species has recently gained new attention as an important environmental weed.

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APPENDIX -

COMPENSATION IN PHOTOSYNTHESIS IN *B. DAVIDII* AFTER A DEFOLIATION-INDUCED REDUCTION IN LIGHT INTERCEPTION

APPENDIX -

COMPENSATION IN PHOTOSYNTHESIS IN *B. DAVIDII* AFTER A DEFOLIATION-INDUCED REDUCTION IN LIGHT INTERCEPTION

A 1 Introduction

Compensation in response to defoliation often includes an increased photosynthetic rate in leaves (Hoogesteger and Karlsson, 1992; Vanderklein and Reich, 1999; Hart *et al.*, 2000; Thomson *et al.*, 2003; Retuerto *et al.*, 2004). *B. davidii* not only exhibits a high phenotypic plasticity but also a high photosynthetic capacity and a highly efficient resource capture and utilisation in general (Feng *et al.*, 2007). The positive relationship between maximum photosynthetic rate and foliar N content is well recognised (Field *et al.*, 1983; Field and Mooney, 1983; Kuppers *et al.*, 1988; Hull and Mooney, 1990) and there is evidence that the photosynthetic nitrogen use efficiency (PNUE) can be improved by increased allocation of N to the photosynthetic apparatus (Evans, 1989; Poorter and Evans, 1998; Niinemets *et al.*, 2003). As an upregulation of photosynthesis is often seen in plants after artificial defoliation (Hoogesteger and Karlsson, 1992; Meyer, 1998; Vanderklein and Reich, 1999; Hart *et al.*, 2000), it was hypothesised that this maybe the case in *B. davidii* also, especially so as a significant increase in foliar N was observed in defoliated buddleia plants in comparison to undefoliated plants (Chapter 4). In this study investigations were first made into the possibility of upregulation of photosynthesis in pre-existing leaves after defoliation treatment.

A 2 Methods

A 2.1 Measurements of photosynthesis

The experimental site and set up, climate, species and defoliation treatment has been described in detail in Chapter 2, so that only the details concerning measurements of photosynthesis are described here. At the end of summer of the first growing season, measurements of photosynthesis were made on one fully-expanded leaf on each of 12 randomly chosen plants per treatment using a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA). The plants were watered on the evening before the measurement day to assure that they experienced no drought stress. To investigate instant up-regulation after defoliation, photosynthesis was measured at ambient CO_2 (370 ppm) and light conditions on two days before (7 and 8 February 2005) the defoliation treatment (9 February) and on the day thereafter (10 February 2005). To investigate long term- changes in photosynthesis, light response and CO_2 response curves (A/ c_i) were taken three to five days before (from 4-6 March 2005) defoliation was applied (9 March) and five to eight days thereafter (from 14-17 March 2005). The light response was measured at 370 ppm CO_2 at a photosynthetic photon flux density (PPFD) of 2000, 1500, 1000, 500, 200, 150, 100, 75, 50 and 0 µmol m^{-2} s^{-1}. The CO_2 response was measured at saturated irradiance (2000 µmol m^{-2} s^{-1}PPFD) at CO_2 partial pressures of 1500, 1200, 800, 600, 450, 370, 250, 200, 150, 100, 50 and 0 ppm. Leaf temperature ranged from 20-28°C, averages of the relative humidity in the leaf chamber ranged from 60 -78%.

Plate A.1 Measuring photosynthesis on 3 month-old buddleia plants.

A 1.2 Data analysis

The light response data were fitted after the standard method of (Prioul and Chartier, 1977) using Photosynthesis Assistant (Dundee Scientific) to calculate Q_E, R_d, Q_o and Q_{sat} while values of A_{max} were obtained from averaging the two highest values measured. To obtain J_{max} and V_{cmax} the A/ c_i data were fitted after Leuning using Sigma Plot (Systat Software, 2002). All statistical analysis was carried out using SAS (SAS Institute, 1996). Variables were tested for assumptions of normality and heterogeneity of variance and no transformations were necessary. A mixed model with block as random effect was applied to detect treatment differences and interaction of defoliation and time.

A 3 Results

Although values for A_{max}, Vc_{max} and J_{max} were slightly higher in defoliated plants (Table A.1 and A.2), the treatment differences were not significant nor was there an interaction of defoliation and time for any of the observed photosynthetic parameters (Table A.1, 2, 3). No evidence of upregulation of photosynthesis in pre-existing leaves, neither in the short term (Table A.3) nor long term (Table A.1 and A.2), was observed after defoliation.

Table A. 1 Light response of defoliated and undefoliated plants measured at ambient CO_2 after the 3rd defoliation in the first growing season in March (end of summer) 2005. Shown are means ± 1 SE (n = 12) of the maximum photosynthetic capacity (A_{max}), the quantum efficiency (Q_E), the calculated dark respiration (R_d), the irradiance at light compensation (Q_o) and the irradiance at saturated A_{max} (Q_{sat}) five to three days before (time 1) and five to eight days after (time 2) defoliation treatment. Using a mixed model, no significant treatment differences or interactions of treatment and time at $P < 0.05$ were found for any of the parameters.

	Time 1		Time 2	
	Defoliated	Control	Defoliated	Control
A_{max} (μmol m^{-2} s^{-1})	19.02 ± 0.84	18.20 ± 1.18	23.00 ± 1.02	21.19 ± 1.17
Q_E (mol mol^{-1})	0.044 ± 0.002	0.055 ± 0.004	0.045 ± 0.003	0.048 ± 0.003
R_d (μmol m^{-2} s^{-1})	1.36 ± 0.19	1.62 ± 0.28	0.71 ± 0.13	0.75 ± 0.10
Q_o (μmol m^{-2} s^{-1})	30.90 ± 3.98	28.46 ± 3.82	15.12 ± 1.94	15.33 ± 1.55
Q_{sat} (μmol m^{-2} s^{-1})	544.1 ± 34.7	444.9 ± 48.1	642.1 ± 25.2	544.5 ± 24.3

Table A. 2 CO_2 –response at saturated light in defoliated and undefoliated plants measured after the 3rd defoliation in the first growing season in March (end of summer) 2005. Shown are means ± 1 SE (n = 12) of the maximum carboxylation rate (Vc_{max}) and the maximum rate of the photosynthetic electron transport (J_{max}) five to three days before (time 1) and five to eight days after (time 2) defoliation treatment. Using a mixed model, no significant treatment differences or interactions of treatment and time at $P < 0.05$ were found for any of the parameters.

	Time 1		Time 2	
	Defoliated	Control	Defoliated	Control
Vc_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	65.8 ± 4.2	60.6 ± 6.2	72.0 ± 2.8	69.0 ± 3.9
J_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	153.4 ± 12.0	155.5 ± 11.3	167.1 ± 6.3	160.5 ± 9.5

Table A. 3 Photosynthesis at ambient CO_2 and light conditions in defoliated and undefoliated plants measured one (time 1) and two (time 2) days before and one (time 3) day after the 3rd defoliation in March 2005 (end of summer in the first season). Shown are means ± 1 SE (n = 12) of the maximum photosynthetic capacity (A_{max}), the stomatal conductance (g_s), intercellular CO_2 –concentration (c_i) and the ratio of extracellular and intracellular CO_2 –concentration (c_i/ c_a). Using a mixed model, no significant treatment differences or interactions of treatment and time at $P < 0.05$ were found for any of the parameters.

	Time 1		Time 2		Time 3	
	Defoliated	Control	Defoliated	Control	Defoliated	Control
A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	12.73 ± 1.63	13.24 ± 1.84	22.74 ± 0.20	21.03 ± 0.75	14.64 ± 3.51	20.60 ± 0.12
g_s ($mmol\ m^{-2}\ s^{-1}$)	361.3 ± 85.0	396.2 ±78.9	857.5 ± 22.2	635.0 ± 56.6	505.8 ± 116.6	559.9 ± 24.9
c_i ($\mu mol\ mol^{-1}$)	196.3 ± 42.3	224.7 ± 34.6	286.1 ±1.6	272.9 ± 3.5	190.3 ± 46.8	270.2 ± 3.9
c_i/ c_a	0.80	0.76	0.78	0.74	0.77	0.73

A 4 Discussion

Responses of plants to defoliation often include stimulation of growth through increases in carbon fixation. This can be achieved by either increasing photosynthetic capacity per unit leaf area (Hoogesteger and Karlsson, 1992; Meyer, 1998) or increases in light interception (Anten *et al.*, 2003; Watt *et al.*, 2007) or both (Caldwell *et al.*, 1981; Pinkard *et al.*, 2007). In this study, no significant treatment difference in any of the measured parameters of photosynthesis was observed. Maximum values of A_{max}, J_{max} and Vc_{max} of pre-existing leaves were not different between defoliated and undefoliated plants. This means that up-regulation of photosynthesis per unit leaf area in pre-existing leaves in response to defoliation was not evident in *B. davidii*, either as an instantaneous response or in the longer term. This is surprising, as there was evidence that defoliated buddleia plants significantly increase foliar N, especially in new leaves, (Chapter 4, Fig. 4.4) compared to undefoliated plants. Hikosaka *et al.* (2005) observed similar increases of foliar N after defoliation in *Quercus serrata*. Today, the positive correlation between photosynthetic capacity and foliar N content is widely accepted (Field and Mooney, 1986; Evans, 1989; Hikosaka, 2004) and increases in photosynthesis with increased foliar N well documented (Kuppers *et al.*, 1988; Hull and Mooney, 1990). Recently, Feng *et al.* (2007) observed in field grown, undefoliated *B. davidii* that a much higher allocation of N to the photosynthetic apparatus was responsible for their significantly higher photosynthetic capacity and photosynthetic nitrogen use efficiency (PNUE) when compared with other fast growing woody species. This would suggest that an increment in foliar N (if the portion of N allocated to photosynthesis had likewise increased) should result in increased photosynthetic capacity.

It is necessary to understand, however, that measurements of photosynthesis were taken early in the first season (after the third defoliation) so that the plants may not have experienced the full impact of the two-season defoliation treatment. In pine and larch an effect on photosynthesis has been noted only in the second year after defoliation (Vanderklein and Reich, 1999). Caution must be taken when linking results of photosynthetic measurements to the data of nitrogen translocation, as photosynthesis was measured in the first season but N translocation (i.e. harvest and analysis of N contents) was investigated in the second season. Therefore, it is possible, that high levels of foliar N in old (pre-existing) leaves were not yet present when photosynthesis was measured. To fully understand the impact of defoliation on photosynthesis in *B. davidii*, measurements should be repeated during the growing period to include seasonal variations of photosynthesis. Also, new grown leaves (those that developed under the influence of defoliation) should be included to examine possible long-term adjustments in photosynthesis. This, however, was outside the scope of this study and could be addressed in future research. No upregulation of photosynthesis in pre-existing leaves was observed, but further study is instructive to ascertain that defoliation did not influence photosynthesis in new leaves.